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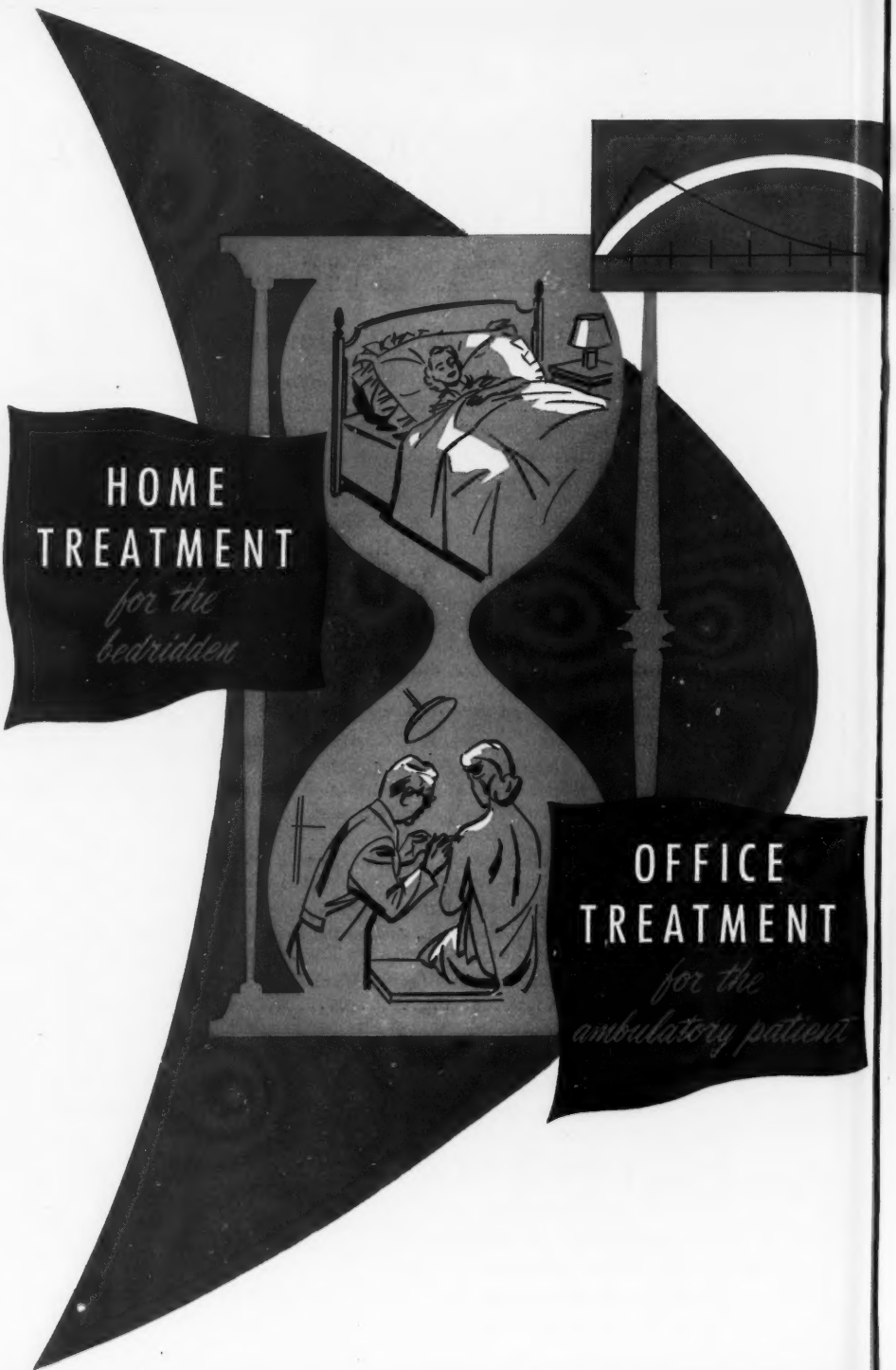
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The Journal of
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VOLUME 12 JANUARY, 1952 NUMBER 1
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COMPARISON OF EFFECTIVENESS OF THREE TESTOSTERONE PREPARATIONS IN MAN AND RATS

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SUBSTITUTIVE therapy of endocrine deficiencies attempts, as far as possible, to emulate the normal secretory activity of the gland in question. In the treatment of eunuchoidism this has been reasonably accomplished by injection of testosterone propionate in oil (1) or of testosterone in aqueous suspension (2). Both these preparations have a rather protracted activity and therefore the need for multiple injections is reduced. Recently another testosterone preparation, testosterone β-cyclopentylpropionate (TCP), was made available to us for clinical trial and it was deemed advisable to compare its effects with those of the propionate and the aqueous suspension, in the therapy of male hypogonadism. Its androgenic activity was also tested in laboratory animals.

METHODS AND RESULTS

Urinary 17-ketosteroid excretion following testosterone propionate in oil and testosterone in aqueous suspension

In the first part of this investigation, 7 patients with primary hypogonadism were given testosterone propionate in oil initially and later, an equal dose of aqueous suspension of testosterone with an interval of from four to twelve weeks between each administration. Six patients (Cases 1

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* Now located in Ottawa, Ontario, Canada.

through 6) received a single intramuscular injection of from 100 to 300 mg. of each of the preparations, with no allowance made for the difference in molecular weight of the two compounds. In Case 1 the dose in both instances was 100 mg.; in Cases 2, 3, 4 and 5, the dose was 200 mg. each, and in Case 6 it was 300 mg. Another patient (Case 7) received both preparations in four doses of 50 mg. each, over a period of six days.

The following are brief records of Cases 1 through 7:

Case 1. A patient 19 years of age was observed with primary prepuberal hypogonadism and accompanying cryptorchidism and gynecomastia, probably Klinefelter's syndrome. Gonadotropin excretion (3) was 424 and 636 mouse units per twenty-four hours and 17-ketosteroid excretion (4) was 3.7 mg. per twenty-four hours.

Case 2. A 29-year-old man had idiopathic prepuberal pituitary failure with associated small stature, hypometabolism, and hypogonadism. Urinary 17-ketosteroid excretion was less than 1 mg. per twenty-four hours.

Case 3. A 55-year-old man manifested primary hypogonadism, mild in degree. Gonadotropin excretion was normal to high, in several assays. The total sperm count was 11,000,000. Symptoms of generalized weakness, and decrease in libido and potentia improved during testosterone propionate therapy at an average dose level of 30 mg. per day.

Case 4. A 30-year-old man was observed with primary prepuberal hypogonadism. Gonadotropin excretion was 318 to 424 mouse units per twenty-four hours and 17-ketosteroids, 5.1 mg. per twenty-four hours.

Case 5. Prepuberal idiopathic hypogonadism in a man, aged 40, had been observed over a period of many years. The patient had been maintained on testosterone preparations. When therapy was discontinued the urinary gonadotropin titer was between 13 and 52 mouse units per twenty-four hours and 17-ketosteroid excretion was 5.4 mg. per twenty-four hours.

Case 6. The patient, aged 53, had primary hypogonadism, mild in degree. Gonadotropin excretion was within normal range, and the 17-ketosteroid excretion was 2.4 mg. per twenty-four hours. His symptoms suggested "male climacteric" and included failing potentia and hot flashes. No treatment with androgens had been undertaken prior to the test doses.

Case 7. A 23-year-old man was observed with primary prepuberal hypogonadism and cryptorchidism. Gonadotropin excretion exceeded 424 mouse units per twenty-four hours and 17-ketosteroid excretion was 3.7 mg. per twenty-four hours.

Results are graphically represented in Figures 1 through 7.

Therapeutic effectiveness of testosterone cyclopentylpropionate as compared with testosterone propionate: 17-ketosteroid excretion following administration of these compounds

This part of the study was carried out in an attempt to evaluate a newly developed testosterone ester which, in animal experiments (5), appeared to have certain advantages over both aqueous suspension of testosterone and testosterone propionate in oil.

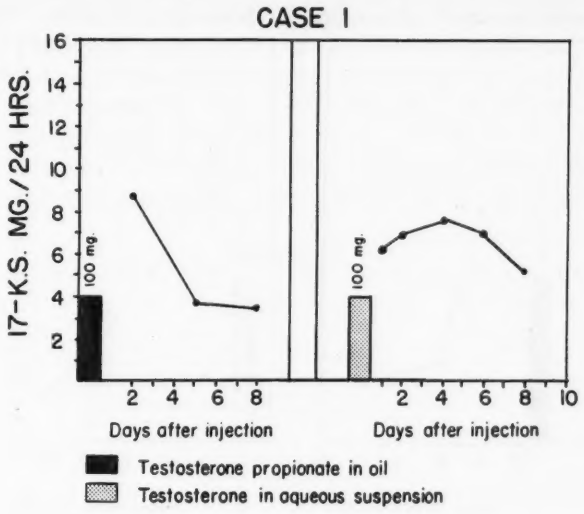
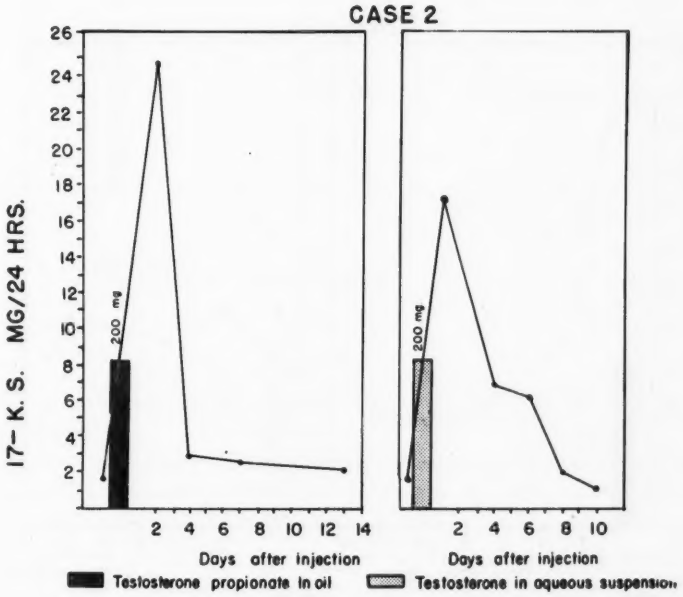


FIGURE 1



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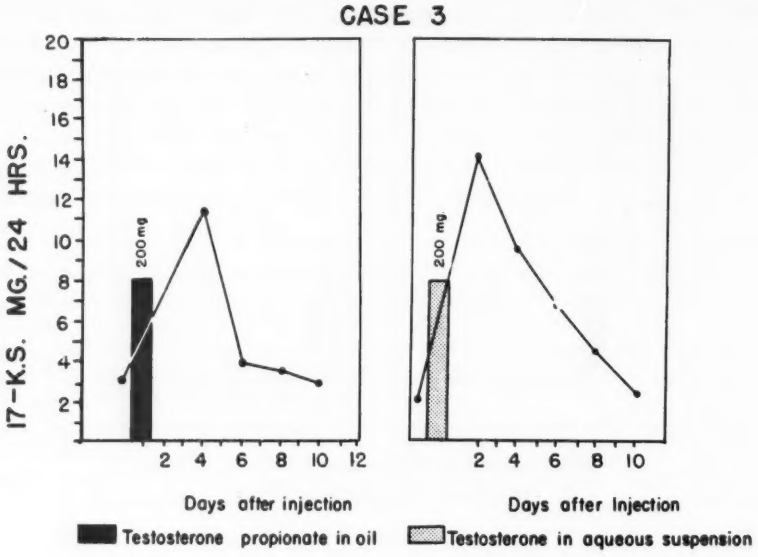

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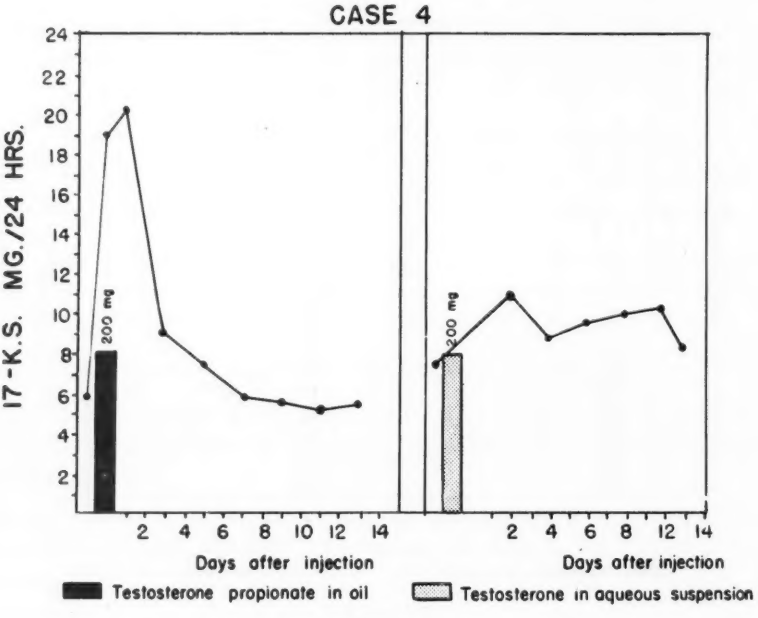

FIGURE 4

Four men with hypogonadism were used. None of them had been previously employed in the studies on testosterone propionate and aqueous testosterone. In 3 of the 4 cases hypogonadism was secondary to pituitary failure, so that presumably there was a deficiency of adrenocortical androgen as well as testicular androgen.

In each instance a single dose of 300 mg. of testosterone propionate in oil was given intramuscularly and approximately four weeks later, a

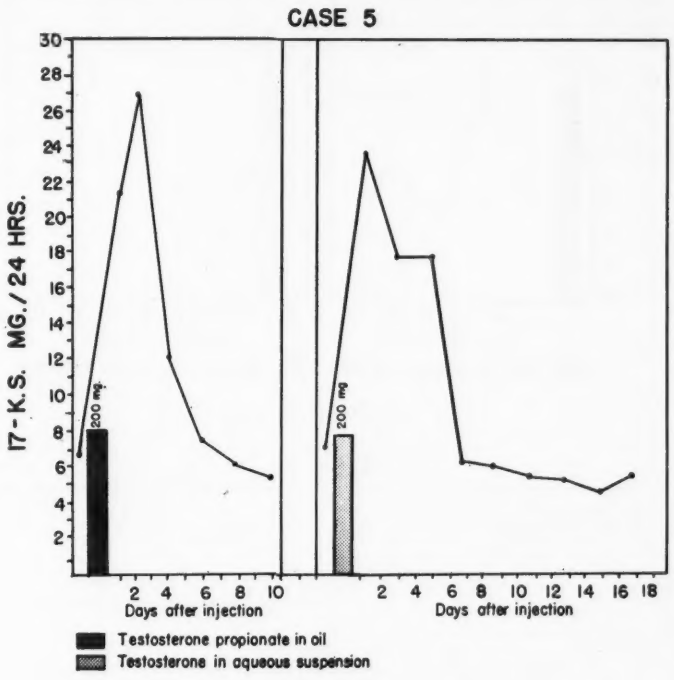

FIGURE 5

single dose of 300 mg. of testosterone cyclopentylpropionate in oil was given intramuscularly.

In Cases 8 and 9 the 17-ketosteroid excretion rates were the only data systematically recorded. However, in Cases 10 and 11 semen volumes and fructose concentration were measured in order to obtain an indication of the effect on end-organ function. In addition, an effort was made to measure the fluctuations in libido and potentia. Semen fructose determinations were made by an adaptation of the method of Harrison (6) for the determination of inulin.

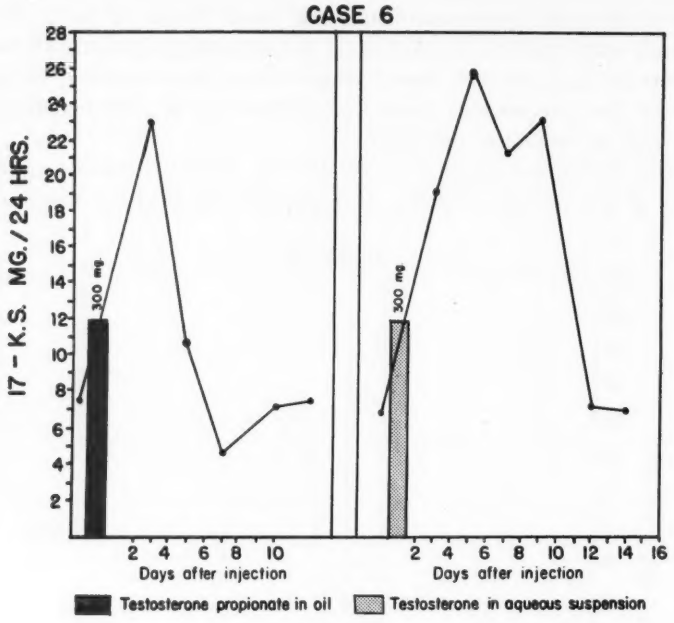

FIGURE 6

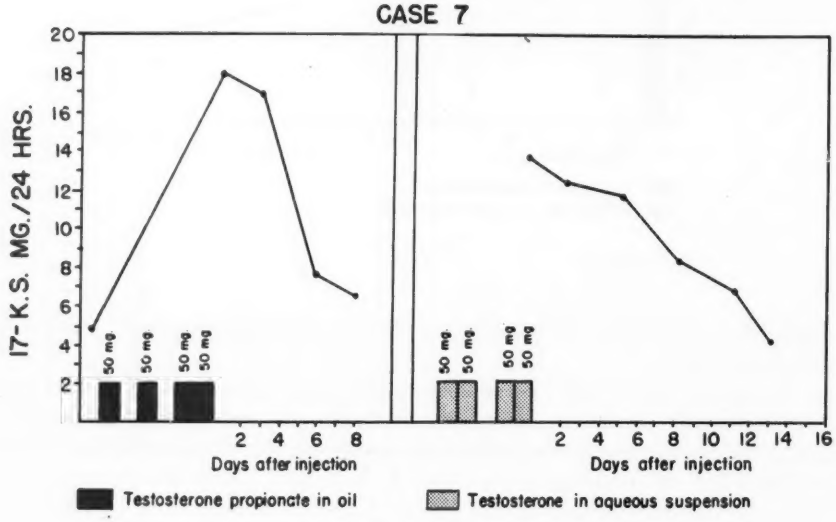

FIGURE 7

Protocols:

Case 8. A 69-year-old man showed severe hypogonadism of twenty-six years' duration, which had followed removal of a suprasellar cyst. The daily maintenance dose of 30 mg. of buccal testosterone propionate was discontinued ten days prior to collection of the control specimen of urine for 17-ketosteroid assay. Subjective effects of the test doses of testosterone propionate and testosterone cyclopentylpropionate were nearly identical in this case. There was an increase in the number of erections, beginning two to three days after each injection and gradually diminishing over a period of from two to three weeks. The only other effect of therapy which this patient experienced was relief for two to three weeks from a feeling of languor, which had been one of his chief complaints. Figure 8 graphically represents the 17-ketosteroid excretion.

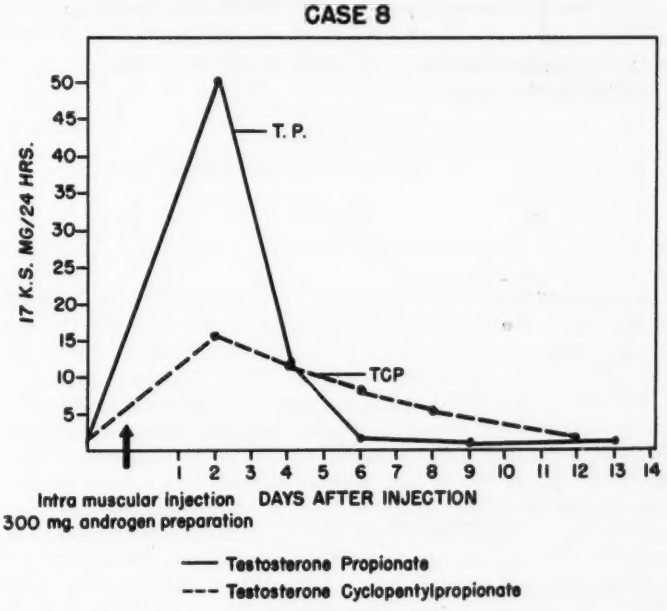

FIGURE 8

Case 9. A patient, aged 20, evidenced pituitary dwarfism and infantilism. Gonadotropin excretion was less than 6 mouse units per twenty-four hours and 17-ketosteroid excretion less than 1 mg. in twenty-four hours. During therapy with chorionic gonadotropin and testosterone propionate considerable improvement occurred over a period of three years. He was maintained later on testosterone propionate—125 mg. intramuscularly twice a week. The last dose was given nine days prior to the collection of the control specimen of urine. This patient reported a more prolonged effect on potentia, libido, and appetite after testosterone cyclopentylpropionate than after testosterone propionate. After administration of the latter steroid the intense libido and increased potentia between the third and seventh days after injection impressed the patient as an unpleasant feature of therapy. After testosterone cyclopentylpropionate, these effects were less in-

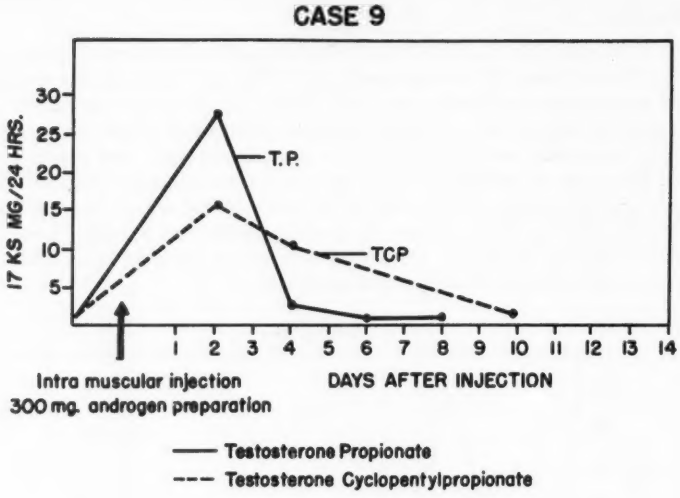

FIGURE 9

tense but persisted at a satisfactory level for as long as four weeks. The excretion of 17-ketosteroids is shown in Figure 9.

Case 10. A man 43 years of age had moderately severe anterior pituitary insufficiency, probably the result of a chromophobe adenoma which expanded the sella turcica. Gonadotropin excretion was less than 6 mouse units in twenty-four hours and 17-ketosteroid excretion, 0.8 mg. per twenty-four hours. The Kepler water excretion index was 2.2 and response to the ACTH test was subnormal. During testosterone propionate therapy considerable improvement in weight, strength, libido and potentia resulted. Later a dose of 30 mg. per day of testosterone propionate was administered by buccal absorption. This therapy was discontinued ten days before a control specimen of urine was collected, preparatory to injection of TCP. Measurements of semen volume, semen fructose and urinary 17-ketosteroid were carried out at intervals for about three weeks after each of the test doses of androgen. A daily record was kept by the patient in an attempt to estimate libido roughly from 0 (absent) to 4 plus (intense); and potentia as well, in terms of erections per day. These data are tabulated in Figure 10. There were no changes in semen fructose attributable to the difference between the two testosterone preparations; fructose values ranged from 41.5 to 58.5 mg. per 100 cc.

Case 11. A 33-year-old male with primary hypogonadism was observed because of delayed puberty. He had been treated with A.P.L. and testosterone over a period of many years and recently had been receiving testosterone propionate, 50 mg. intramuscularly three times per week. This therapy was discontinued eight days before the first test dose of 300 mg. of testosterone propionate. Twenty-four days elapsed between the giving of testosterone propionate and the giving of cyclopentylpropionate. Qualitatively no sharp distinction was noted by the patient between their effects. However, the duration of the activity of the cyclopentylpropionate exceeded that of the propionate by more than a week. The subjective and objective effects of the treatment which lend themselves to quantitative measurement are recorded in Figure 11. Semen fructose concentration ranged from 210 to 540 mg. per 100 cc.

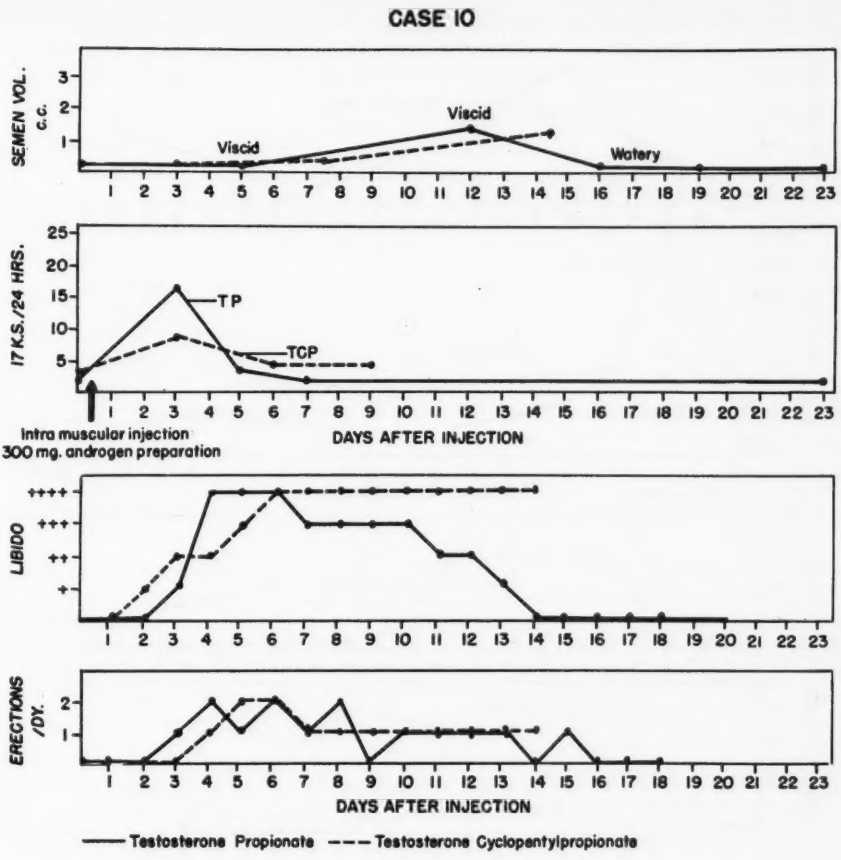

FIGURE 10

Effect of testosterone cyclopentylpropionate, testosterone propionate, and testosterone in aqueous suspension on the sex accessories of the castrated male rat

Forty-five male rats were castrated when 21 days of age. They were then divided into 3 groups of 15 each, and received testosterone in the three different forms, *viz.*, the aqueous solution, the propionate, and the cyclopentylpropionate. A single subcutaneous injection of 1 mg. of testosterone per rat (calculated as free testosterone) was given to the animals a week after castration and 5 rats from each of the 3 groups were sacrificed on the third, sixth and eighth days after castration. Weights of prostate and seminal vesicles are recorded in Table 1.

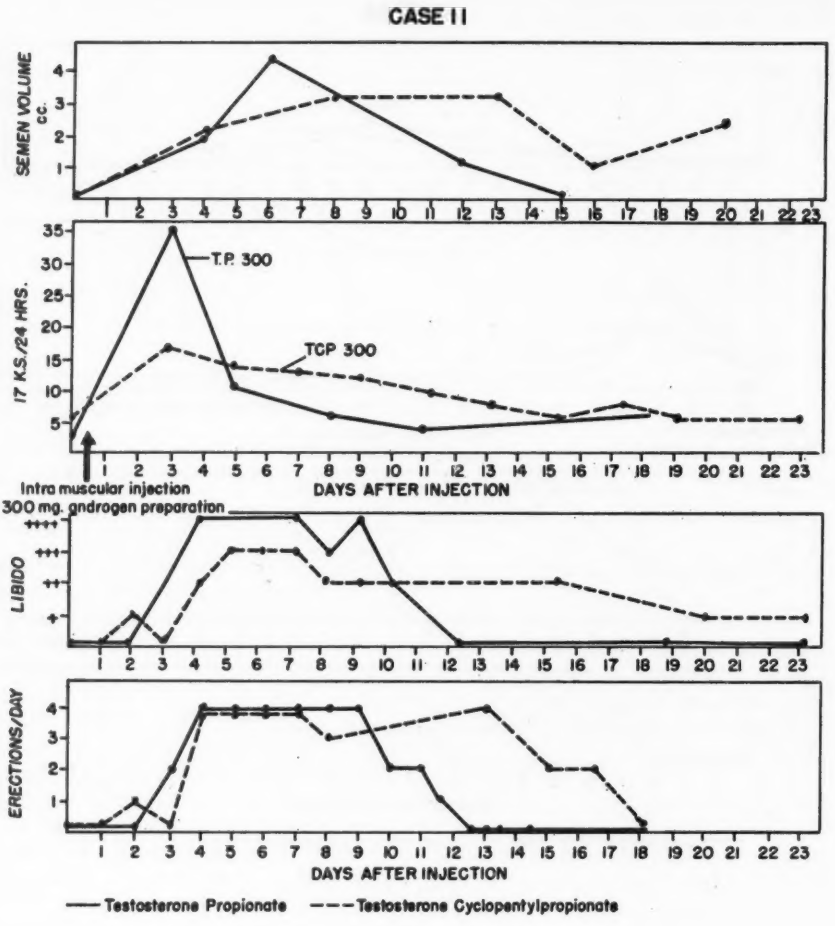

FIGURE 11

DISCUSSION

It is apparent from the preceding observations that 17-ketosteroid excretion is ostensibly more protracted after administration of the aqueous suspension of testosterone than after testosterone propionate. These data are in keeping with the findings of Sevringhaus and Sikkema (2) who found that aqueous suspensions of testosterone are as least as effective as testosterone propionate in the therapy of male hypogonadism. More recently Hamburger (7), and Hamburger and Kaae (8), comparing the excretion rate of 17-ketosteroids after injection of testosterone crystal

TABLE 1. EFFECT OF THREE TESTOSTERONE PREPARATIONS ON THE WEIGHT OF PROSTATE AND SEMINAL VESICLES IN THE IMMATURE CASTRATE RAT*

Testosterone preparation	3rd Day after injection		6th Day after injection		8th Day after injection	
	Prost., (Gm.) mg	Sem. ves. (Gm.) mg	Prost., (Gm.) mg	Sem. ves. (Gm.) mg	Prost., (Gm.) mg	Sem. ves. (Gm.) mg
Testosterone cyclopentyl-propionate	74.0	38.7	107.2	83.2	133.9	108.4
Testosterone propionate	68.4	34.5	79.9	46.4	63.1	38.7
Aqueous testosterone	56.0	24.0	54.9	40.2	101.2	75.9

* For further explanation, see text.

suspensions and testosterone propionate in oil, found by this criterion that the oil preparation was excreted largely within the first twenty-four hours, whereas the crystal suspensions had a slow excretion. In the 4 patients described in our communication, the excretion of 17-ketosteroids following injection of the new preparation, testosterone cyclopentyl-propionate, was similar to that following injection of the crystal suspensions.

With respect to libido and potentia, the effect of a single dose of testosterone propionate is, generally speaking, more intense and of shorter duration than that following a single injection of testosterone cyclopentylpropionate.

The experiments performed on rats confirm the findings of Sakamoto *et al.* (5), who found a greater and more prolonged anabolic and androgenic effect with testosterone cyclopentylpropionate than with testosterone propionate or with testosterone aluminum phosphate. With the small doses employed by us the effects of testosterone propionate were already vanishing while the effects of aqueous testosterone, and especially of the cyclopentylpropionate, were still increasing.

The values reported here for semen fructose in 2 of the patients who were azoospermic do not deviate appreciably from those reported for fertile individuals by Harvey (9). The normal variations in semen fructose concentration make the estimation of this substance an unsuitable method of evaluating androgenic activity. However, in the azoospermic person, in whom the intervening factor of sperm activity metabolizing fructose is

eliminated, the concentration of fructose in semen increases proportionally to the amount of androgen administered (unpublished observations of the authors).

SUMMARY

Judging from the curves of urinary 17-ketosteroid excretion, it appears that, in man, testosterone in aqueous suspension has a somewhat more prolonged effect than an equal dose of testosterone propionate in oil.

The excretion of 17-ketosteroids following administration of testosterone cyclopentylpropionate is considerably more prolonged than that following an equal dose of testosterone propionate.

The effect of a single injection of testosterone cyclopentylpropionate on semen volume, libido, and potentia in the men described, appeared to be less intense and more prolonged than that following an equal dose of testosterone propionate.

Experiments in castrated male rats showed that the duration of androgenic activity is more prolonged after testosterone cyclopentylpropionate than after free testosterone or testosterone propionate.

Acknowledgments

This investigation was supported partly by a grant to the Department of Endocrinology, made by Mr. Dean Francis.

We are indebted to Dr. H. F. Hailman, of the Upjohn Co., Kalamazoo, Michigan, for the testosterone cyclopentylpropionate.

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TESTOSTERONE β-CYCLOPENTYLPROPIONATE: A NEW LONG-ACTING ANDROGEN

ARNOLD C. OTT, PH.D., MARVIN H. KUIZENGA, PH.D., STANLEY C. LYSTER AND BYRON A. JOHNSON, B.S.

From The Research Laboratories of The Upjohn Company, Kalamazoo, Michigan

THE general acceptance of testosterone propionate by the parenteral route for therapy of androgen deficiencies is based primarily on early studies with several esters of testosterone. After the early initiation of this work by Ruzicka (1, 2), the intensity and prolongation of androgenic response to many esters was investigated (3, 4, 5). None of these was found to be of significantly greater activity than the propionate, which became generally accepted as the most effective form of testosterone. Miescher, Wettstein and Tschopp (6) reported that aliphatic short-chain fatty acid esters of testosterone effected greater seminal vesicle growth response in young castrate rats than did either free testosterone, or aliphatic long-chain fatty acid esters. The palmitate and stearate produced only minimal, if any, androgenic response. Recently Mooradian, *et al.* (7) have reported that certain alkoxy and alkylmercapto acyl esters of testosterone have somewhat higher activity than testosterone propionate when immediate response to injection of the test materials was observed in castrate rats. Data concerning the duration of activity of these derivatives were not presented.

That the enhanced activity of testosterone propionate over free testosterone is due to slower utilization of the androgen itself from the injection site was indicated by the findings of Kochakian. He showed that frequent injections of testosterone produce a response which approaches that produced by testosterone esters (8).

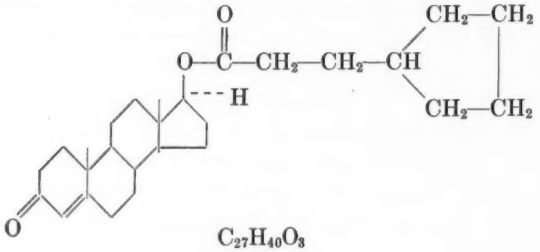
Much attention has, therefore, been given to obtain a more ideal androgenic response by utilizing repository forms of microcrystalline testosterone, since such a preparation administered either subcutaneously or intramuscularly should be ideal for slow absorption and should produce as good or better response than injection of an oil solution of testosterone propionate. However, our own laboratory experiments, designed to evaluate the activity of aqueous suspensions of microcrystalline testosterone in comparison with the propionate, as judged by seminal vesicle response in both immature and castrate rats, have failed to demonstrate any superior androgenic effect for the aqueous suspensions. Clinical results indicate that aqueous suspensions may be at least as effective as testosterone propionate in oil (9). It appears, therefore, from evidence available to date that no injectable androgen preparation presently available has significant

Received for publication August 13, 1951.

advantages over the oil solution of testosterone propionate, the favorable effects of which were shown by McCullagh in the therapy of testicular deficiency (10). It seemed desirable, however, to continue the testing of testosterone derivatives with the objective of finding a preparation which would have definite advantages over both the oil solution of the propionate or the aqueous suspension of free testosterone. For maximum therapeutic usefulness, such an androgen should effect both immediate and prolonged response, so that uniformity of replacement therapy can be attained with a minimum of injections.

The early work of Deanesley and Parkes (11) also showed that it is desirable to obtain maximum uniformity of testosterone utilization in order that no abnormal relationship of prostate growth to seminal vesicle growth results. These investigators noted that androsterone caused an abnormal ratio of growth between the prostate and the seminal vesicles, the prostate size being always much greater than that of the seminal vesicles. Frequent injections of testosterone or the use of oil solutions of the esters resulted in greater growth of the seminal vesicles compared with that of the prostate. The rate of growth of these accessory organs was more nearly that which exists in the normal state with intact testes, if testosterone propionate were used instead of the other androgens, or free testosterone. The seminal vesicle and prostate growth responses are, therefore, useful in evaluation of new androgens for the purpose of developing a better therapeutic agent—one that, after single injection, would bring about the most prolonged period of response together with early onset of effect, and not differ significantly from normal testicular secretion in effect on seminal vesicles and prostate.

The androgen which we believe exhibits the ideal properties to far greater extent than even testosterone propionate, is the new derivative, testosterone β-cyclopentylpropionate, herein also designated as TCP and represented as:



$C_{27}H_{40}O_3$

M.W. = 412.59
m.p. 101–102°C.
$[\alpha]_D^{25}$ +87 ($CHCl_3$)

This androgen was selected from a series of carboxylic acid esters of testosterone and was further compared with free testosterone and testosterone propionate by various modes of administration.

EXPERIMENTAL

The types of acyl esters of testosterone which were tested are represented by the 15 examples listed in Table 1. The acids were converted to their respective acid chlorides, which were subsequently made to react with testosterone in the normal manner. The resulting esters were isolated and then dissolved in the appropriate vehicle, for bioassay. Details for the synthesis of testosterone β-cyclopentylpropionate and other testosterone acyl esters have been reported (12).

For the evaluation of androgenic potency and duration of response, young male rats of the Sprague-Dawley strain were used. All animals were kept in air conditioned rooms with temperature controlled at 78° ± 2° F. and relative humidity at 45 per cent. Both immature intact rats and immature castrated rats were used. The former were employed to screen the series of compounds in order to select those with the highest degree of androgenic potency in comparison with testosterone propionate. For more accurate comparison of effects on both seminal vesicles and prostate, and evaluation of duration of activity from a single injection, the immature castrated rats were used.

The new compounds were first evaluated, therefore, by injection subcutaneously in a dose of 5 mg. into 26- to 28-day-old male rats. The total dose was given in one injection. All esters were tested as solutions in cottonseed oil at a concentration of 5 mg. per cc. The aqueous suspensions of testosterone were injected in a dose of 5 mg. in 0.2 cc. of saline. Beginning the third day after injection and every third day thereafter until the eighteenth day, 3 to 5 animals were sacrificed, the seminal vesicles and prostates were removed, carefully dissected free of other tissues and their weights recorded. The technique for dissection of these tissues was the same as described by Hays and Mathieson (13). At least 18 rats were used for the screening of each compound.

Although the immature rat with intact testes is satisfactory for assay of the propionate and other esters having effects which disappear before the animal's own glands become active, continued effect beyond the eighteenth day, as is characteristic of the cyclopentylpropionate, makes it essential to use the castrate rat for comparison of maximum extent and duration of activity. Young male rats were castrated from 26 to 28 days after birth. Four weeks after castration, testosterone or a derivative was administered in a single dose equivalent to 5 mg. of free testosterone. For each prepara-

TABLE 1. SEMINAL VESICLE AND PROSTATE GROWTH RESPONSE TO TESTOSTERONE ESTERS IN IMMATURE MALE RATS. (All compounds injected subcutaneously in a dose of 5 mg. on the 1st day.)

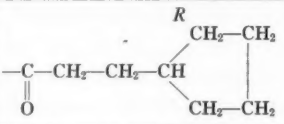
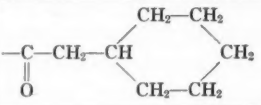
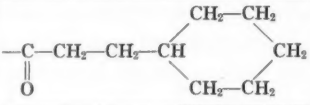
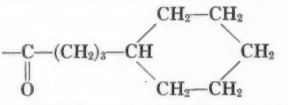
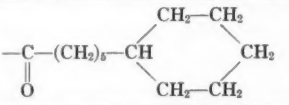
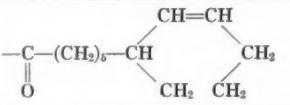
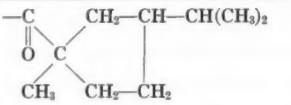
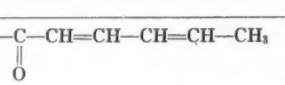
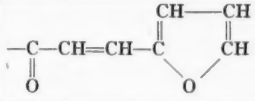
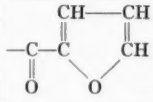
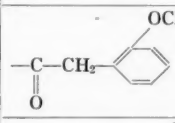
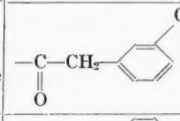
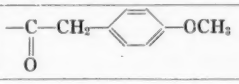
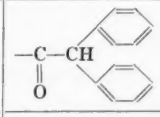
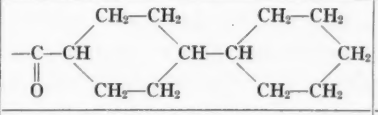
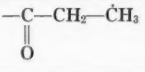
Compound	Structure	Androgenic response (wt. in mg.)					
		12th day*		15th day		18th day	
		Sem. ves.	Prostate	Sem. ves.	Prostate	Sem. ves.	Prostate
1. Testosterone β-cyclopentyl-propionate		321	343	323	360	444	423
2. Testosterone cyclohexyl-acetate		99	235	66	210	124	229
3. Testosterone β-cyclohexyl-propionate		299	390	378	349	150	311
4. Testosterone γ-cyclohexyl-butyrate		152	307	206	340	267	379
5. Testosterone ε-cyclohexyl-caproate		101	206	52	178	69	219
6. Testosterone ε-(Δ^3-cyclo-hexenyl) caproate		62	186	74	216	72	215
7. Testosterone 1-methyl-3-isopropyl-cyclopentyl-1-carboxylate		16	90	29	140	46	157
8. Testosterone sorbate		94	222	124	256	101	219

TABLE 1. SEMINAL VESICLE AND PROSTATE GROWTH RESPONSE TO TESTOSTERONE ESTERS IN IMMATURE MALE RATS. (All compounds injected subcutaneously in a dose of 5 mg. on the 1st day.)—*Continued*

Compound	Structure	Androgenic response (wt. in mg.)					
		12th day*		15th day		18th day	
		Sem. ves.	Pros-tate	Sem. ves.	Pros-tate	Sem. ves.	Pros-tate
9. Testosterone β-(2-furyl) acrylate		142	305	121	254	83	232
10. Testosterone furane-2-carboxylate		74	256	159	288	182	329
11. Testosterone *o*-methoxy-phenylacetate		36	142	54	195	74	200
12. Testosterone *m*-methoxy-phenyl acetate		31	122	39	164	62	181
13. Testosterone *p*-methoxy-phenyl acetate		51	167	63	192	72	199
14. Testosterone α, α-diphenyl acetate		35	132	36	161	56	190
15. Testosterone 4-bicyclohexyl carboxylate		34	143	35	132	72	179
16. Testosterone propionate		184	334	255	387	81	231

* After first injection.

tion, at least 50 rats were used, 5 animals being sacrificed every third day after injection until completion of the experiment on the thirtieth day.

RESULTS

In immature intact rats

The seminal vesicle and prostate growth response for the types of testosterone esters investigated are given in Table 1 for 15 specific examples. Since the primary interest was in obtaining a compound with prolonged activity, only the responses on the twelfth, fifteenth, and eighteenth days after administration of the androgen are presented. The results with testosterone propionate are tabulated for comparison. It is of great interest that the introduction of the cyclopentyl moiety on the terminal carbon of the propionyl group of testosterone propionate greatly enhanced the activity. This compound (No. 1 of Table 1), the cyclopentylpropionate of testosterone, is the most active of the series tested. It is apparent that the cyclohexylpropionate possesses a similar degree of activity, with a definite difference, however, in favor of the cyclopentyl moiety. It may be concluded that the introduction of the cyclic grouping onto the propionyl group greatly enhances the activity and also duration of action.

The effect of decreasing or increasing the length of the carbon chain between the steroid nucleus and the cyclic moiety is apparent from the data recorded for the cyclohexylacetate (compound 2), butyrate (compound 4) and caproate (compound 5). None of these produced as great a response as the cyclopentylpropionate. Compounds with other cyclic structures were likewise of lower degrees of activity. Aliphatic esters of testosterone have previously been made and tested in comparison with the propionate in other laboratories and found to have little or no advantage (6). That unsaturated aliphatic esters would likewise have no advantage over the propionate is indicated by the results obtained with the sorbate (compound 8).

It may be argued that increased response and prolongation of effect might better be obtained with a repository of microcrystalline testosterone, subcutaneously or intramuscularly. This should be ideal for slow absorption and, therefore, produce as good or better response than oil solutions of the better testosterone esters, if the activities of such compounds are influenced merely by the slow rate of hydrolysis of the ester at the injection site. Slow absorption should be attained with microcrystalline suspensions. As may be seen from results presented in Table 2, aqueous suspensions of free testosterone have in our hands given disappointing results. Comparison of the effect of testosterone as a microcrystalline suspension in saline (single injection of 5 mg. in 0.2 cc.) was made with testosterone propionate, both by single injection of the latter in a single dose of 5 mg. and by ad-

ministration over ten days of a total of 5 mg. in doses of 0.5 mg. per day. It is apparent that at no period after injection of aqueous testosterone suspension was the response greater than for testosterone propionate. It is also interesting to observe that the expected enhanced response, as shown on the eighteenth day, to repeated injections of testosterone propionate was still no greater than the response to a single injection of an equal total dose of TCP as shown for the eighteenth day in Table 1. This result again indicates the superiority of TCP over any form in which testosterone was administered.

TABLE 2. ANDROGENIC RESPONSE TO TESTOSTERONE AQUEOUS SUSPENSION AND TESTOSTERONE PROPIONATE IN OIL IN THE 26- TO 28-DAY-OLD RAT

Material	Dose	Androgen response (wt. in mg.)												Total No. of rats ves.
		3rd day*		6th day		9th day		12th day		15th day		18th day		
		Sem. ves.	Prostate	Sem. ves.	Prostate	Sem. ves.	Prostate	Sem. ves.	Prostate	Sem. ves.	Prostate	Sem. ves.	Prostate	
Testosterone, aqueous suspension	5 mg. in 1 injection	21 (3 rats)	118	57 (3 rats)	200	131 (3 rats)	349	98 (3 rats)	201	59 (4 rats)	190	42 (3 rats)	159	19
4LG6 Testosterone propionate in oil	5 mg. in 1 injection	18 (3 rats)	124	48 (3 rats)	186	136 (3 rats)	346	242 (3 rats)	445	191 (4 rats)	371	351 (3 rats)	436	19
Testosterone propionate in oil	5 mg. in 10 injections of 0.5 mg. per day							321 (3 rats)	383	371 (2 rats)	565	450 (2 rats)	555	7
Control	No injection	8 (1 rat)	62	11 (1 rat)	86	11 (1 rat)	71	9 (1 rat)	68	11 (1 rat)	75	16 (1 rat)	90	6

* After first injection.

Since the response to TCP was greater on the eighteenth or last day of the experiments with uncastrated immature rats, the question arises as to when maximum response becomes evident. Experiments were, therefore, completed with immature intact male rats in which observations were made every third day until the thirtieth day; the results showed that TCP was still effective on the thirtieth day. As is well known, however, the seminal vesicle and prostate growth responses beyond the eighteenth day are influenced by endogenous production of androgen by the rat's own maturing testes, the activity of which was manifest in the growth of the accessory sex organs beginning the twenty-fourth day of the experiment, or about the fiftieth day of the rat's life (14, 15). The continued effect of TCP beyond the eighteenth day made it essential to use the castrated rat for comparison of extent and duration of activity.

In immature castrated rats

Results of experiments using the castrated rat are recorded in Table 3. Groups of at least 5 rats were sacrificed every three days beginning the third day after subcutaneous administration of the androgen, until completion of the experiment on the thirtieth day. For comparison of prolonged activity, the responses after the twelfth day are most significant; therefore, the responses for the third, sixth and ninth days are not shown in Table 3. The androgenic response, as measured by seminal vesicle and

TABLE 3. SEMINAL VESICLE AND PROSTATE RESPONSES TO TESTOSTERONE β-CYCLOPENTYLPROPIONATE (TCP), TESTOSTERONE PROPIONATE, AND AQUEOUS SUSPENSION OF TESTOSTERONE IN CASTRATED RATS, COMPARED WITH RESPONSES TO ENDOGENOUS ANDROGEN

Compound	Dose (mg. eq. of testosterone) in single injection	Androgenic response (wt. in mg.)							
		12th day		18th day		24th day		30th day	
		Sem. ves.	Prostate	Sem. ves.	Prostate	Sem. ves.	Prostate	Sem. ves.	Prostate
Testosterone β-cyclopentylpropionate	5 mg. in oil	478 (15 rats)	468	555 (15 rats)	594	528 (15 rats)	610	392 (15 rats)	563
Testosterone propionate	5 mg. in oil	141 (10 rats)	275	72 (10 rats)	186	57 (10 rats)	155	47 (10 rats)	118
	25 mg. in oil	531 (5 rats)	527	674 (5 rats)	704	443 (5 rats)	581	219 (5 rats)	390
Testosterone	5 mg. in 0.2 cc. aq. susp.	209 (5 rats)	280	43 (5 rats)	107	27 (5 rats)	91	39 (5 rats)	109
Normal controls with intact testes		540 (5 rats)	475	683 (5 rats)	589	818 (5 rats)	795	817 (5 rats)	774
Castrated controls. No injections		10 (10 rats)	45	9 (10 rats)	51	9 (10 rats)	40	11 (10 rats)	37

prostate weights, for the complete 30-day postinjection period are graphically presented, however, in Figure 1. It is apparent that by removal of the animal's own maturing testes, the remarkable difference in activity of the cyclopentylpropionate and propionate becomes most obvious. As shown in Figure 1, response to either of these esters of testosterone was equally rapid, but on the twelfth day the response to the new ester, TCP, was already triple that to an equal dose of the propionate. This increased response became tenfold from the eighteenth to the thirtieth day after injection. Only when the dose of testosterone propionate was increased five times over that of the cyclopentylpropionate was the duration and magnitude of the response comparable to that observed with TCP. Even the 25-mg. dose of testosterone in the form of the propionate did not equal the

response to 5 mg. in the form of the cyclopentylpropionate on the twenty-fourth and thirtieth days. This intensive and prolonged action of TCP in the castrated rat has been independently confirmed in both McCullagh's and Gordon's laboratories.

Although 15 rats were used for each point on the curve for TCP in Figure 1, there is some degree of irregularity. There can be, however, no question of the significance of the difference between the responses to the two esters, since the areas covered by the individual responses after the

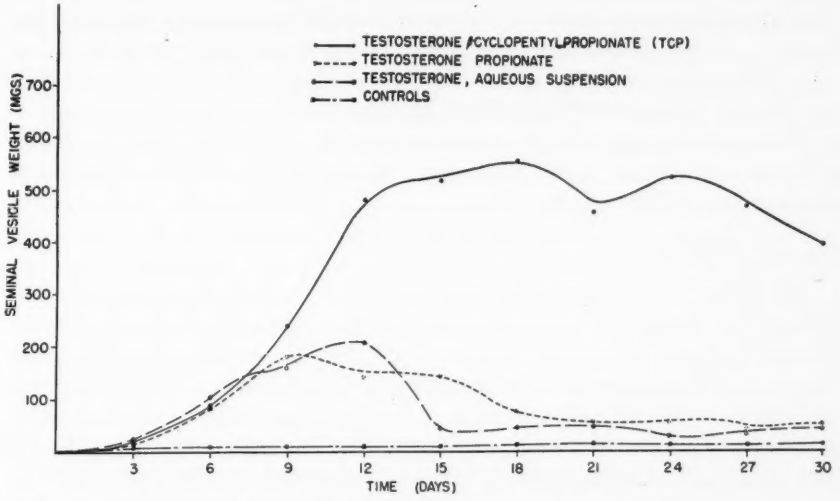


FIG. 1. Testosterone β-cyclopentylpropionate—comparison with other androgen preparations administered by injection. (Vehicle: cottonseed oil, each cc. of which, for a single-dose injection, contained the testosterone ester in an amount equivalent to 5 mg. of testosterone.)

Microcrystalline testosterone, 5 mg. in 0.2 cc. of saline, was given in a single-dose injection.

ninth day are far removed from each other, and particularly after the twelfth day, there is no overlapping of individual responses.

It is interesting to note also that in castrated rats, free testosterone in aqueous suspension gave about the same degree and duration of effect as the propionate. These results agree better with the clinical data (9) than do the results in uncastrated rats.

In Table 3, we have also tabulated the weights of the seminal vesicles and prostate of uninjected normal control rats of the same age and same dates of birth as the injected rats. It is noteworthy that the single administration of the TCP was able to stimulate seminal vesicle and prostate

growth so that by the eighteenth day it was practically the same as the growth produced by the animal's own testicular secretion. This is all the more remarkable when it is considered that the results of castration had been given four weeks to become manifest, and that the weight of the accessory organs at the time of injection was far below that of uncastrated controls. For example, the average weight of the seminal vesicles of the castrate rats at the time of injection was about 10 mg., whereas that of littermate noncastrates was about 500 mg.

It may also be noted from the data in Table 3 that a more normal relationship of prostate growth to seminal vesicle growth was produced by TCP than by testosterone propionate. The seminal vesicle growth in the case of the intact rats slightly exceeded prostate growth. With testosterone propionate, seminal vesicle growth lagged far behind prostate growth. TCP more nearly simulated the normal relationship, particularly on the twelfth and eighteenth days—a further indication of a more uniform and optimum androgen response.

Results of toxicity tests

Testosterone propionate has been used for several years and is undoubtedly a safe therapeutic agent; it must, therefore, be shown that testosterone β-cyclopentylpropionate is no more toxic than the propionate. The question arises as to whether the liberation in the organism of β-cyclopentylpropionic acid would cause a greater toxicity than propionic acid.

The sodium salts of β-cyclopentylpropionic acid and propionic acid were administered to rats in aqueous solution in doses of 100, 200, 400, 800 and 1,600 mg. per Kg. subcutaneously. A group of 5 rats was used at each dose level. No impairment of growth was observed following administrations of either compound over an 11-day period after injection. The growth at the lowest dose level was 65 Gm. for the cyclopentylpropionate and 49 Gm. for the propionate; and at the highest dose level, 48 and 55 Gm. respectively. Local irritation with necrosis at the site of injection was apparent only at the 800-mg. and 1,600-mg. dose levels, with both substances. The lesion was most severe with the 1,600-mg. dose of sodium β-cyclopentylpropionate but was completely healed in ten days.

Since in humans the usual dose will not be in excess of 100 mg. per injection, which could yield at most only 35 mg. of free β-cyclopentylpropionic acid, there is little chance of local irritation by this new acyl group.

An attempt to determine the LD_{50} of TCP was unsuccessful because the highest concentrations attainable in oil solutions were still not sufficient to allow the administration of enough of the substance to cause systemic toxic effects. Both TCP and testosterone propionate in a dose of 1,600 mg. per Kg. subcutaneously produced no toxic effects, either systemic or local, in rats weighing 200 Gm. at the time of injection.

Ten rabbits were used in a chronic toxicity experiment. Four of them (2 males and 2 females) were given 30 mg. of TCP three times weekly for four weeks. Another group of 4 (2 males and 2 females) were given 25-mg. doses of the propionate, being equivalent in testosterone content to the 30-mg. dose of the cyclopentylpropionate. Two animals (1 male and 1 female) served as controls. Body weight was recorded once a week. The average total weight gains of the animals were 440 Gm. for those receiving TCP, 405 Gm. for those receiving testosterone propionate, and 365 Gm. for the 2 controls. On the thirtieth day all the animals were sacrificed and tissue specimens of heart, liver, lung, spleen, adrenal glands, testes, seminal vesicles, prostate, bone, ovaries, uterus, vagina and pituitary gland were submitted for histopathology.

No pathologic changes were apparent on gross examination, except those attributable to the hormonal action of the administered substance on the sex organs. The ovaries of the control female, for example, weighed 320 mg.; the average weight of these glands in the 2 females which had received TCP was 130 mg.; and in those animals given testosterone propionate, the weight of the ovaries was 170 mg. This atrophic effect on female sex organs was apparent also in the uterus and the vagina.

In the male rabbits, following administration of either ester, there was atrophy of the testes as indicated by a 50 per cent reduction of the size of these glands. The seminal vesicles and prostates, however, were larger than in the controls, due to response to the large dose of androgen.

Microscopic examination of the tissues for pathologic changes revealed no significant "morphologic alterations in the tissues" examined, attributable to toxicity.[1] "Apparent hyperplasia" was noted for those sex organs which are known to be affected by androgen therapy, and was associated with increased weight of these same male accessory organs as just described. In 1 of the female rabbits an adrenal lesion was noted. This animal had received testosterone propionate and it is questionable whether this isolated finding of adrenal damage is significant. No damage, however, was noted with the new ester, TCP. Atrophy of interstitial cells of the ovary was found following both TCP, and testosterone propionate.

It is well known that any inherent toxicity of a compound becomes more apparent in adrenalectomized than in normal animals. This has been observed for testosterone propionate in experiments designed primarily to determine whether androgen could substitute for the life-maintaining activity of the adrenal cortex. No such activity was found, but on the contrary, a decrease in survival time in rats was reported by Spurr and Kochakian (17), who observed that 5 mg. per day of testosterone propionate resulted in a survival time of 3.8 days as compared to 8.7 days for

[1] We are grateful to Dr. E. S. Feenstra of these laboratories for the pathologic studies.

a similar group of controls. This was interpreted as a decidedly toxic effect. A "toxic effect" as measured by thymus involution in adrenalectomized animals was also reported by Schacher, Brown and Selye (18).

Because of these results, we compared the effects of the new ester, TCP, and testosterone propionate in adrenalectomized rats. Thirty male rats were adrenalectomized when 28 days old. The average survival period of 10 controls was 7.8 days; in a parallel group of 10 rats this value was reduced to 5.7 days by daily administration of 5 mg. of testosterone propionate, and to 5.7 days in the group of 10 receiving the cyclopentylpropionate in a dose of 5 mg. per day. Obviously, therefore, TCP is no more toxic than testosterone propionate, even in adrenalectomized animals.

SUMMARY AND CONCLUSIONS

A series of testosterone derivatives has been made and screened for intensity and duration of androgen effect in the immature intact rat, as determined by seminal vesicle and prostate growth response. On the basis of this test, testosterone β-cyclopentylpropionate (TCP) has been selected.

Further study of the new androgen in comparison with testosterone propionate and aqueous suspension of free testosterone in castrated rats has disclosed that it produces greater and more prolonged effects. The seminal vesicle growth responses on the eighteenth to thirtieth days after administration are about ten times those obtained with testosterone propionate. These results indicate that this new androgen, found to be so much more active than testosterone propionate by the identical test which resulted in the extensive development of the therapeutic use of the latter, is an advance toward ideal parenteral androgenic therapy.

Having shown by acute and chronic toxicity studies that the cyclopentylpropionate is no more toxic than the propionate and, therefore, safe for human studies, clinical work has been undertaken. Preliminary reports indicate that TCP in man has an androgenic effect which is several times more prolonged than that of testosterone propionate (19, 20), using such criteria as changes in 17-ketosteroid excretion, libido, potentia, seminal vesicle fluid volume, and physical strength.

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RELATIVE DURATION OF ACTION OF NATURAL AND SYNTHETIC ESTROGENS ADMINISTERED PARENTERALLY IN WOMEN WITH ESTROGEN DEFICIENCY

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THE duration of action of estrogens administered parenterally has been the subject of systematic research in test-animals, but no analogous systematic study of the duration of action of these substances has been undertaken in women. A certain degree of prolongation of the effect of estrogens was achieved early by esterification of the substances concerned (1–5), and more recently a further lengthening of the action has been achieved by employing improved microcrystalline suspensions (6) or emulsions (7). Only a few such preparations have been studied in women, and seldom have the effects of two or more preparations been compared in the same subjects. The studies on duration of action of estrogen which have been reported in the past have been based chiefly on the suppression of "hot flushes," but the value of these data, based as it is upon a subjective response, has been disputed (8). In tests of this type, however, estradiol dipropionate proved to have more prolonged action than estrone (9) and estradiol-3-benzoate (10, 11). McGavack and associates (12, 13) have shown that a study of the "follicular reaction" of vaginal smears of women does not provide a ready means for determination of the duration of action of a standard dosage of estrogen.

The duration of action of microcrystalline suspensions of estrone has been studied by Freed and Greenhill (14). Von Wattenwyl (15) observed more rapid, more intensive and prolonged estrogenic effects after administration of microcrystalline suspensions of estradiol-3-benzoate than after administration of estradiol dipropionate in oil solution. Toppozada (16) confirmed these results. Stilbestrol dipalmitate was found to be superior to the dipropionate and to nonesterified stilbestrol (Freed, Eisin and Greenhill (17)). Finally, Bloom (18) observed a considerably prolonged estrogenic action after the injection of large doses of dimethoxystilbestrol (45–90 mg. in three weeks).

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METHOD

In order to avoid the criticism which must be met when experiments are based on subjective responses, we confined ourselves exclusively to objective tests. As it was impossible to make our tests on a large number of subjects, we have, in order to eliminate possible variations due to the individual factors, administered successively the greatest possible number of estrogen preparations to every one of the available subjects.

The subjects were:

1) ovariectomized women whose uteri had been preserved (Cases 01, 02, etc.);

2) women suffering from ovarian agenesis (Cases Am 1, Am 6, Am 7, etc.); and

3) women with secondary amenorrhea of varying etiology, always with pronounced and persistent estrogen deficiency.

In order to measure the duration of estrogenic action, we have determined the time interval between cessation of estrogen therapy and the onset of the withdrawal bleeding which commonly takes place when the humoral estrogen falls below a certain level (19, 20). We have termed this interval the "latent period," and it is obvious that this measures only the duration of action on the endometrium; other tissues with a different threshold may show a longer or shorter estrogenic effect. It is interesting to note here that in monkeys considerable variation in dosage had no effect on this time interval ("latent period").

The majority of these patients were subjected to regular studies of the genital tract, especially an estimation of the quantity and degree of viscosity of the cervical mucus (21), and also a study of the "vaginal formula," which is an expression of the percentage of karyopycnotic cells and of cells with cytoplasmic granules (22, 23). In this way, an appraisal was made of the estrogen level in the organism. The possibility should be taken into account of an extinction of the estrogenic effect without the induction of bleeding, a phenomenon which occurs when the fall of the estrogenic level in the blood is gradual and slow (24). On the other hand, in certain cases, absence of bleeding might be a result of subthreshold estrogenic action, due to pronounced retardation of absorption from the site of injection.

We refrained from studying the endometrial response by means of biopsy, so as to avoid a precipitation of withdrawal bleeding.

Two schemes of treatment have been applied as follows:

(a) esters of the estrogen in doses of 5 mg. were given by injection four times, with intervals of three days between injections; and

(b) emulsions and suspensions of natural estrogens in doses of 10 mg. were injected twice in a period of nine days. All injections were made in the gluteal muscle.

The interval between the day of the last injection and the first day of the withdrawal bleeding is termed here the "latent period" ("L.P.").

DISCUSSION

As a rule, the esters of estradiol in oil solution have the same duration of action in women as they have in test-animals. They can be classified according to increasing duration of action, *viz.*, 3-benzoate, dipropionate, and 17-n-butyrate-3-benzoate. The same relative duration of action has been obtained when these have been tested in female rats, whether the criterion employed was the Allen-Doisy test or the uterine weight (3). The 17-caprylate has been found equally interesting, having a long duration of action in brown Leghorn cocks (16).

Among the esters of diethylstilbestrol in oil solution, the n-dibutyric ester should be specially mentioned as having a long duration of action. In animals the n-dibutyric ester of estradiol also has an interesting duration of action (3). The n-butyric radicle seems to have a special tendency to retard in an adequate manner the absorption of estrogens in human subjects as well as in animals.

The aqueous emulsion of estradiol 3-benzoate and the microcrystalline suspensions of estradiol 3-benzoate or of diethylstilbestrol difuroate show particularly long duration of action.

The data on the relative duration of action of various estrogens obtained

TABLE 1

Single standard 10-mg. dose	Duration of action (number of days hot flushes were absent after treatment)		
	Case 1	*Case 2*	*Case 3*
Estradiol 3-benzoate (oil solution)	10		
Estradiol 17-n-butyrate-3-benzoate (oil solution)	30		
Estradiol 17-caprylate (oil solution)	48	40	15
Estradiol 3-benzoate (aqueous emulsion)	49 73	25	25
Diethylstilbestrol difuroate (small granules in oil)	45	45	45
Estradiol 3-benzoate (microcrystalline suspension)	84 82	45	
Methyl ester of 3-methylbisdehydrodoisynolic acid (oil solution)	3	12	

by studying the reactions of the endometrium could probably be applied also to other receptors. The duration of suppression of vasomotor troubles, and more particularly the hot flushes, after administration of the various estrogen preparations varied roughly in the same way as the latent periods before withdrawal bleedings, as can be seen in the figures of Table 1, which shows the incidence of hot flushes recorded after treatment of 3 castrated and hysterectomized women.

SUMMARY

The duration of action of various estrogen preparations was studied. The method employed was to administer by injection a standard total dosage of 20 mg. of each estrogen to women who had been castrated or who suffered from ovarian agenesis, but whose uteri remained intact. The duration of the time interval between the last injection and the onset of withdrawal bleeding was then observed. This was regarded as a measure of the duration of action of the estrogen injected, since with the dosage employed, withdrawal bleeding ordinarily should have occurred unless withdrawal was exceedingly gradual, possibly after a prolonged action. Other studies were made, such as on the changes in the vaginal smears, the character of the cervical mucus, and also the patients' symptomatic responses, in order to detect additional evidence of estrogenic action.

The estrogens which were tested are listed in the order of increasing duration of action. Those with shortest action, as shown by shortest interval between last injection and onset of bleeding are listed first, *viz.:*

a. Estradiol 3-benzoate, estradiol 3-furoate, diethylstilbestrol dipropionate and diacetate, in oil solution.

b. Estradiol dipropionate in oil solution.

c. Estradiol 17-caprylate and estradiol 3-benzoate 17-n-butyrate, in oil solution.

d. Diethylstilbestrol n-dibutyrate in oil solution, diethylstilbestrol difuroate in small crystal oil suspension.

e. Estradiol 3-benzoate in aqueous emulsion.

f. Estradiol 3-benzoate in microcrystalline aqueous suspension and diethylstilbestrol difuroate in large crystal oil suspension.

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The estradiol 3-benzoate was supplied by Roussel Laboratories, through the courtesy of Dr. Gueutier.

The aqueous emulsion of estradiol 3-benzoate was supplied by Organon Laboratories, through the courtesy of Drs. Tausk and Everse.

The estradiol dipropionate, 17-caprylate, 3-benzoate-17-n-butyrate, in oil solution

and the microcrystalline aqueous suspension of estradiol 3-benzoate were supplied by Ciba, Limited, through the courtesy of Dr. Meier.

PROTOCOLS

The time interval (days) between the last injection of estrogen and the onset of withdrawal bleeding (termed latent period, L.P.):

A. NATURAL ESTROGENS

I. In Peanut Oil

1. *Estradiol 3-benzoate*

Case	01	02	03	04	06*
L.P.	8	9	7	8	5

* An endometrial biopsy was performed on the 3rd day after the last injection.

Case	Am 1	Am 2	Am 2*	Am 3	Am 4	Am 5	Am 6	Am 7	Am 8
L.P.	8	8	9	9	7	8	7	9	8

* After 4 times 2 mg. of the same ester; the L.P. was 9 days.

2. *Estradiol dipropionate*

Case	01	02	03	04	05	06	
L.P.	12	12	13	14	16	0Bl*	12

* No bleeding; 8 days after cessation of treatment cervical mucus in moderate quantity had low viscosity; 15 days later cervix was dry.

Case	Am 1	Am 2	Am 3	Am 4	Am 5	Am 6	Am 7	Am 8
L.P.	13	20	16	10	12	15	20	17

3. *Estradiol 3-furoate*

Case	01	02	Am 2	Am 3	Am 4	Am 5
L.P.	7	7	0Bl*	9	8	7

* No bleeding; at the time of the fourth injection the proportion of karyopycnotic cells was 11 per cent; 15 days later, 0 per cent.

4. *Estradiol 17-caprylate*

Case	01	03	04
L.P.	16	14	20

5. *Estradiol 17-n-butyrate-3-benzoate*

Case	01	03	04	Am 1
L.P.	19	15	23	23

II. In Aqueous Emulsion

1. *Estradiol 3-benzoate*

Case	01	02	03	04	Am 1	Am 6
L.P.	29	30	28	0Bl*	34	32

* No bleeding.

III. In Aqueous Microcrystalline Suspension

1. *Estradiol 3-benzoate* (This preparation contained a mixture of two kinds of microcrystals: weight, 0.4γ and 0.14γ; length 0.15 mm. and 0.05 mm.)

Case	01	02	07	Am 1
L.P.	*	76	30**	0Bl***

* Absence of withdrawal bleeding; return of hot flushes after 60 days.

** Dosage only 10 mg.

*** Dosage only 10 mg. (no bleeding).

Cervical mucus of low viscosity was present for at least 54 days. Some supplementary observations showed:—In 1 subject (castrated by x-rays) after 10 mg., the interval was 57 days. In a case of natural menopause, after 10 mg., the interval was 45 days; after two more identical courses of treatment there was no withdrawal bleeding, and hot flushes were suppressed each time for a period of about three months. In another similar case, after 20 mg., bleeding due to glandular hyperplasia occurred from the 40th day after injection; hot flushes were suppressed for 68 days.

B. SYNTHETIC ESTROGENS

I. In Peanut Oil Solution

1. *Diethylstilbestrol diacetate*

Case	01	02	Am 2	Am 3	Am 4
L.P.	10	10	7*	10	7

* After 4 times 10 mg., the interval was 7 days.

2. *Diethylstilbestrol dipropionate*

Case	01	02	03	Am 1	Am 2	Am 3	Am 4
L.P.	8	8	9	10	14	7	0Bl*

* No bleeding. Two days after the last injection the karyopycnotic cells were 50 per cent of the total.

3. *Diethylstilbestrol-n-dibutyrate*

Case	01	02	05	Am 2	Am 4	Am 5
L.P.	25	19	0Bl*	27	17	26

* No bleeding.

In 1 case of castration by x-rays, the interval was 24 days.

II. In Microcrystalline Suspension

1. *Diethylstilbestrol difuroate* was employed in two forms of suspension in peanut oil, as follows:

a. With *large granules,* containing crystals or crystalloid agglomerates, 65 per cent having an average diameter of 13γ, and 35 per cent, 5γ.

Case	01*	Am 2	Am 4**
L.P.	60	44	79

* From the 12th to the 30th day after the last injection, bleeding occurred due to glandular hyperplasia of the endometrium.

** From the 18th to the 30th day after the last injection, bleeding occurred due to glandular hyperplasia of the endometrium.

b. With *small granules*, containing crystals having a maximum diameter of 5 microns

Case	01	02	03
L.P.	25	24	23

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THE EOSINOPHIL RESPONSE TO STRESS OF PATIENTS WITH SURGICALLY PRODUCED THALAMIC AND HYPOTHALAMIC LESIONS*†

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SPIEGEL, Wycis and their coworkers have adapted the Horsley-Clarke stereotaxic apparatus so that precisely localized lesions may be produced within the brain substance of humans. In this manner, they have been treating patients who have certain emotional disorders and intractable pain (1, 2). Consequently, a unique opportunity has been afforded the present authors to investigate the relationship of the thalamus and hypothalamus to the human metabolic economy. Patients so treated have been studied over a period of two years with a variety of tests, including those measuring the response of eosinophils to the stress produced by ACTH, epinephrine and insulin. This paper is limited to a report of changes observed with these latter procedures.

METHODS AND MATERIAL

Fifteen schizophrenic patients have been evaluated before and at intervals after bilateral lesions were produced in the thalamus and/or hypothalamus by Drs. Spiegel and Wycis. Several patients were operated on again and the lesions enlarged and/or second lesions placed in other areas. Fasting blood specimens were taken in balanced oxalate, and the test substance (25 mg. of ACTH intramuscularly, 0.3 mg. of epinephrine subcutaneously, or 0.1 unit per kilogram of body weight of crystalline insulin intravenously) administered. Four hours later, a second blood specimen was similarly drawn. True blood glucose levels (3) were deter-

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mined fasting, and at 15, 30, 45, 60, 90 and 120 minutes after the injection of the test dose of insulin. The eosinophil counts of the oxalated blood specimens were made in Fuchs-Rosenthal counting chambers using the phloxine, propylene glycol-water stain of Randolph (4). Two pipettes were used for each blood specimen, and two chambers were filled from each pipette, the final count representing the average of four chambers.

RESULTS

Four patients showed certain changes postoperatively. Their lesions are enumerated in Table 1. These altered responses to stress agents were generally of similar nature in the 4 patients, so that results in only Patients

TABLE 1. DISTRIBUTION OF LESIONS

Patient	*Thalamus* dorsomedial nucleus	*Thalamus* anterior nucleus	*Hypothalamus* posterolateral
1	x		x
2	x		x
3		x	
4	x	x	

Patient #2, in a second operation, had the lesions in the dorsomedial nucleus of the thalamus enlarged and new ones placed in the anterior nucleus of the thalamus.

#1 and #2 are charted (Figs. 1 and 2). Each chart consists of three parts, the top representing the eosinophil response to epinephrine (E), ACTH (A) and insulin (I); the center one, the level of fasting eosinophils; and the lowest, the insulin tolerance tests.

The preoperative eosinophil responses were normal (drops of 50 per cent or more under fasting eosinophil levels) as were most of the responses during the first month after surgery. After this time, however, they became increasingly abnormal, particularly to the stress of adrenalin (Table 2). Attempts to prime the mechanism which causes blood eosinophils to fall, by giving epinephrine daily were unsuccessful. In Patient #2, a fall in fasting eosinophils was observed immediately following the second operation, after which a rise occurred which persisted for six weeks.

Three of the 4 patients showed no significant change in their insulin tolerance tests postoperatively, although Patient #1 may have been insulin-sensitive even before surgery. In Patient #2, on the other hand, increased insulin sensitivity seems to have developed following the first

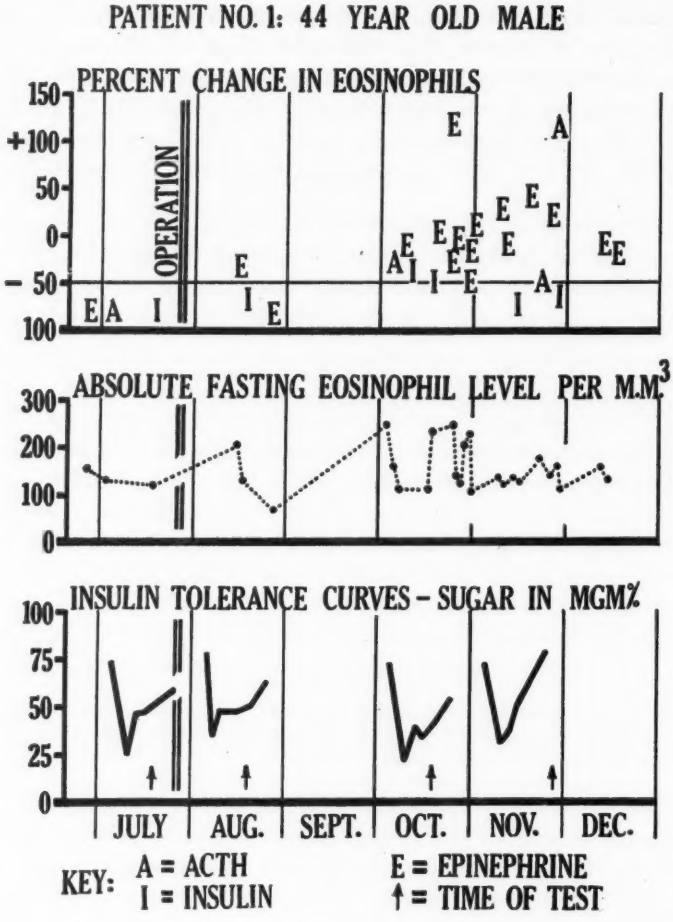

FIGURE 1

operation, and the fourth blood sugar curve (January) suggests hypoglycemic unresponsiveness as well (Fig. 2). Arrows on the graphs signify the time of the month the test was performed.

Alterations in these stress reactions were not generally observed until at least four weeks after surgery. It is, therefore, possible that in others of the 15 patients changes which were overlooked by us may have developed, as the first 11 patients were unfortunately not studied after the first postoperative month.

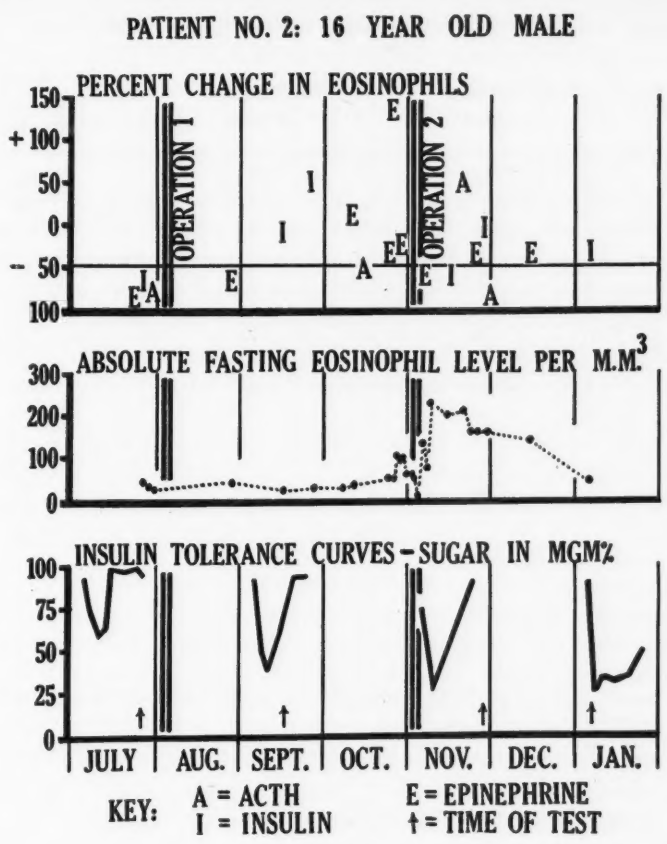

FIGURE 2

DISCUSSION

Known interrelationships between the central nervous system, including the diencephalon, and the endocrine system have been well covered by others (5, 6) and it is not our intention to review this large subject again. Certain references, however, are especially pertinent to the present presentation and will be mentioned briefly.

Hume and Wittenstein (7) found that paramedian lesions in the anterior hypothalamus or at the junction of the middle and posterior hypothalamus in dogs caused a lack of response (in terms of eosinophil drop) to epinephrine and insulin but left the response to ACTH intact. Close scrutiny of their data (their Fig. 6, p. 141), however, shows that postoperatively the eosinophil fall after ACTH was only 40 per cent, whereas it had been

TABLE 2. POSTOPERATIVE EOSINOPHIL RESPONSES

Patient	Stress agent*	Total number of tests	Percentage of abnormal responses
1	E	17	88
	I	5	40
	A	3	67
2	E	5	80
	I	2	100
	A	1	0
3	E	2	100
	I	—	—
	A	1	0
4	E	5	80
	I	2	50
	A	1	0

* E = Epinephrine.
I = Insulin.
A = ACTH.

82 per cent originally. This corresponds to our experience in humans, in whom ACTH occasionally appears to be less effective after surgery. Hume and Wittenstein's animals did not show insulin sensitivity. Only 1 of our 4 patients seemed to show increased insulin sensitivity postoperatively. It may be that the mechanism of eosinophilic response to epinephrine and/or insulin is distinct and separate from that of insulin sensitivity. In this connection, it is interesting to note the drop in fasting eosinophils following the second operation in Patient #2 (Fig. 2), despite marked impairment of eosinophilic response to other modalities of stress. Whether due to the stress of operative trauma or of direct electrical stimulation, it seems to represent another instance of dissociation of responses to stress.

DeGroot and Harris (8) found that electric stimulation of the posterior region of the tuber cinereum or of the mammillary body resulted in lymphopenia in rabbits, similar in magnitude and time relationship to that following emotional stress. The lymphopenia of emotional stress in normal rabbits was abolished or diminished by lesions in the pars tuberalis, in the posterior tuber cinereum, or in the mammillary bodies.

Inasmuch as our patients were schizophrenics, it is important to mention the work of Pincus *et al.* (9), who demonstrated impaired stress responses in many patients of this type. We have also encountered schizo-

phrenics who behave similarly to those reported by Pincus, but the 4 subjects reported in the present paper showed normal responses to stress before operation. The extent to which the choice of patients influences our results, we do not know.

SUMMARY

1. Four of 15 schizophrenic patients showed changes in the eosinophil response to ACTH, insulin and epinephrine following the production of thalamic and hypothalamic lesions by the Spiegel-Wycis apparatus.

2. Lesions in these 4 patients involved the anterior and dorsomedial nuclei of the thalamus and the posterolateral portion of the hypothalamus.

3. Abnormalities appeared four weeks postoperatively; they were greatest when the test material was epinephrine, and least when it was ACTH.

4. Three of the 4 patients showed no change in insulin sensitivity. The fourth showed insulin hypersensitivity and possibly hypoglycemic unresponsiveness. This same patient also displayed a sustained rise in the fasting eosinophil level following a second operation.

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HORMONE-RESISTANT PSYCHOGENIC AMENORRHEA

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FUNCTIONAL amenorrhea resistant to steroid hormone therapy is a known phenomenon although it is not often seen and has not been studied in sufficient detail. We are, therefore, reporting 2 such cases with a study of the mechanism involved.

Case 1. R. W. presented herself in May, 1948, at the age of 33, because of amenorrhea of four and one-half years' duration.

The menarche occurred at about 13 years; menses were fairly regular except for a four-month period of amenorrhea at the age of 17, which occurred during extended travels. Menstruation was re-induced by hormone therapy of an unknown nature. The patient married at the age of 19 and subsequently became pregnant; the pregnancy was voluntarily terminated during the first trimester. Menstruation was fairly regular thereafter. A change of environment at the age of 23 did not affect the menses. At the age of 29 (seven years after a divorce) she underwent severe psychic stress because of an emotional entanglement with a very unstable individual. Amenorrhea developed and persisted for four and one-half years, despite intensive therapy.

Psychic tensions characterized the patient's domestic and personal life from early childhood.

Physical examination revealed a well-proportioned, sthenic habitus with fully developed secondary sexual characteristics. The breasts were of normal size and consistency. The clitoris and external genitalia were normal; the cervix was long, and the uterine fundus conical with a cervico-uterine ratio of 2:1. Both ovaries were somewhat enlarged, firm, but not irregular.

Laboratory data. Repeated blood counts and urinalyses gave normal results, as did determinations of the blood sugar, chloride, urea nitrogen, uric acid, and of the serum cholesterol, calcium, inorganic phosphate, total protein, chloride and sodium. The basal metabolic rate was −3 per cent. The specific dynamic action of proteins was within normal limits. X-ray examination of the skull revealed a normal cranial vault and sella turcica.

Urinary 17-ketosteroid excretion[1] at various times over a two-year period was 3.4,

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[1] Urinary 17-ketosteroids were isolated by acid hydrolysis, ether extraction and purification; the neutral ketonic steroids were separated by means of the reaction with the Girard T reagent and were measured colorimetrically by the Zimmermann reaction according to the absolute-alcohol method of Callow and Callow (1).

Estrogens in the urine were extracted by the method of Engel *et al.* (2) after acid hydrolysis. The estrogenic material was determined fluorometrically according to the method of Bates and Cohen (3), using pure estrone as a standard.

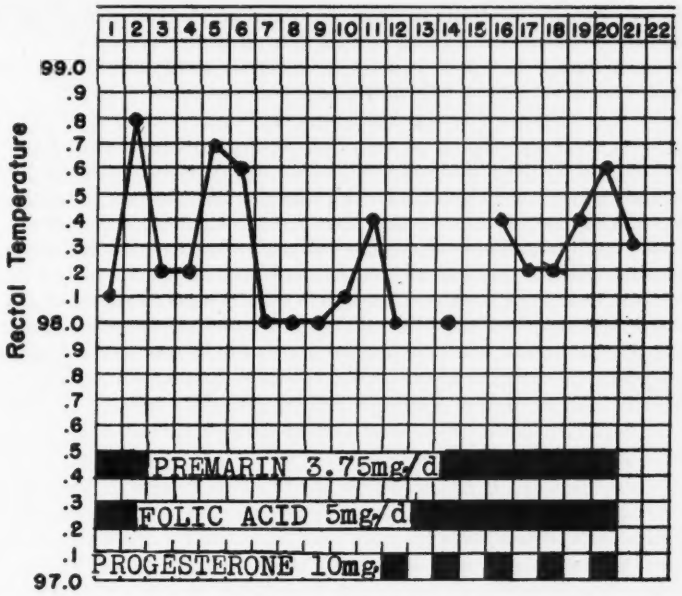

FIG. 1. Basal body temperature curve in Patient #1 during period of amenorrhea Treatment consisted of estrogen, folic acid and progesterone, as indicated by the solid black areas.

7.3, 3.6, and 8.5 mg. per twenty-four hours. The normal range for women of this age is 5 to 12 mg. per twenty-four hours.

Urinary gonadotropins were determined five times during the period of study. The values were: less than 8.0 I.U.; less than 8.0 I.U.; more than 1.3 I.U.; 7 to 10 I.U.; and 3.3 to 6 I.U. per twenty-four hours. The normal range for urinary gonadotropins is 2 to 10 I.U. per twenty-four hours.

Urinary excretion of estrogens was determined fluorometrically on a number of occasions. Several of these urine specimens were also sent to Dr. Lewis L. Engel, whose values agreed closely with ours. The results obtained were: 3.6, 5.2, 5.5, 8.3 and 3.4 gamma per twenty-four hours. These values are extremely low for an adult female: excretion normally ranges from 15 to 40 gamma per twenty-four hours, depending on the time of the menstrual cycle.

The findings of an "estrogen tolerance test" will be discussed subsequently.

An endometrial biopsy specimen consisted of small fragments of tissue. The endometrial

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Gonadotropins were isolated from pooled, refrigerated 48-hour urine samples by the ultrafiltration method of Gorbman (4). The protein thus obtained was assayed by serial dilution in 21-day to 24-day-old CFW mice, using 100 per cent increase in uterine weight as the endpoint. Similar assays were carried out with serial dilutions of international standard pregnant mare serum, and the results of gonadotropin assays were expressed as equivalent to a certain number of I.U. of PMS.

glands were very small and straight; the stroma was scanty and showed considerable infiltration with mononuclear cells and occasional neutrophils. A vaginal smear showed small round epithelial cells with moderately vesicular nuclei; cornification was rare. Neutrophils were infrequent and Döderlein bacilli were present.

Clinical course. Cyclic oral estrogen-progesterone therapy (3.75 mg. of Premarin per day for twenty days plus 30 mg. of anhydrohydroxyprogesterone during the last ten days) produced barely detectable staining for one day. Repeated, intensive therapeutic attempts were equally unsuccessful; a few are listed for their theoretic implications:

1. Premarin (7.5 mg.) for twenty days; anhydrohydroxyprogesterone (30 mg.) during the last ten days. Result: barely detectable staining for two and one-half days; only 50 per cent keratinization of the vaginal cells, and no elevation of basal body temperature.

2. Premarin (3.75 mg.) for twenty days; folic acid (5 mg.) concomitantly; a total of 325 mg. of crystalline progesterone during the last ten days; prostigmine during the last three days. Result: barely detectable vaginal staining for three days; inflammatory reaction caused by the progesterone crystals invalidated the temperature curve.

3. Estrogen and folic acid as just described; progesterone 5×10 mg. in oil during the last ten days. Result: barely perceptible vaginal staining for two days; no significant elevation in basal body temperature (Fig. 1).

4. Estrogen: a total of 29 mg. of estrone in oil for twenty days; a total of 150 mg. of progesterone in oil during the last ten days. The vaginal smear showed moderate numbers of neutrophils; epithelial cells ranged from small to normal in size; some were still ovoid with large vesicular nuclei. Keratinized cells were rare. There was some "baking together" of the cells. An endometrial biopsy specimen consisted of fragments of tissue. These showed small atrophic glands.

5. Ethinyl estradiol, 0.1 mg. for twenty days. Result: no menstrual bleeding. Vaginal smear showed cells of moderate to small size, nearly all unkeratinized, with nuclei generally vesicular.

6. Stilbestrol (5 mg.) for twenty days. Starting four days after withdrawal of medication, there was detectable vaginal bleeding for two days (one intravaginal tampon per day) and staining for an additional two days. On the last day of medication, the vaginal epithelial cells were 90 per cent cornified and showed small pyknotic nuclei.

Eventually an "estrogen tolerance test" was performed. A 48-hour urine was collected after the patient had been without medication for one month. Two mg. of estrone (in oil) was injected daily for two consecutive days (total 4 mg.) and all urine on these days and the day after was collected and pooled. The control daily excretion averaged 1.6 gamma. The total estrogen excretion in the seventy-two hours following injection was 11.3 gamma; thus, of the 4,000 gamma of estrone injected, only 6.5 gamma was recovered in the urine, a yield of 0.16 per cent. This may be compared with numerous studies (5, 6, 7) in which it has been shown that the usual recovery in normal women is of the order of 5 to 10 per cent.

Further course. Some months after these observations and the end of therapy, the patient achieved an emotionally satisfactory romantic tie. Not long afterward, there was a brief episode of spontaneous vaginal staining, and a month later, she experienced a profuse menstrual period requiring three intravaginal tampons daily for a period of two and one-half days. The next menstrual cycle was followed closely with vaginal smears: these showed 5 to 10 per cent cornification in the early phase of the cycle and 60 to 80 per cent cornification in the second half of the menstrual cycle, which was twenty-eight days in length and terminated in menstruation lasting four and one-half days. Spontaneous cycles continued and the basal body temperature curve was diphasic, as shown

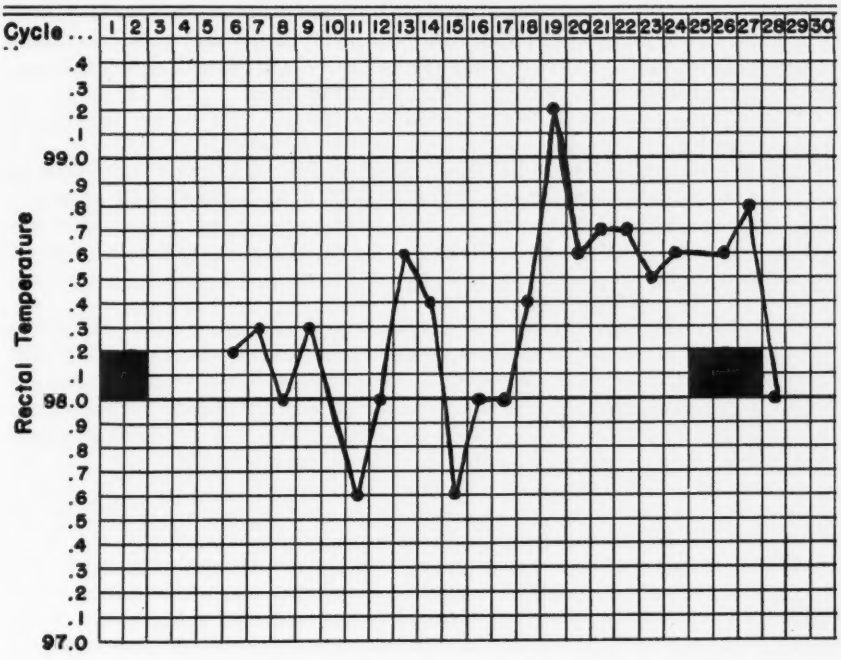

FIG. 2. Basal body temperature curve in Patient #1 during normal menstrual cycle (menstruation indicated by solid black areas).

in Figure 2. For six months there was normal menstruation, but then the romantic tie was abruptly broken and amenorrhea reappeared promptly.

Case 2. F. M. was a 24-year-old nun who consulted us because of progressive weight gain for three years and hypertrichosis of one year's duration. The menarche occurred at 15 years of age. Menstruation had never been regular and was punctuated with periods of amenorrhea lasting up to one year. The spontaneous menstrual period which occurred immediately prior to her consulting us followed an amenorrhea of seven months.

Physical examination revealed a generally obese, but distinctly feminine individual 67½ inches tall and weighing 216 pounds. There were no purple striae. A slight amount of hair appeared on the forearms and lower legs, but there was none on the body except for pubic hair of normal feminine pattern. The face showed increased hair growth on the upper lip, chin and downward along the cheeks. The blood pressure was 132/82. Pelvic examination under anesthesia revealed a minute clitoris, absence of the labia minora, and a relatively small uterus with a well developed cervix. The adnexa were normal. A vaginal smear at this time showed most of the cells to be cornified.

Because of the possibility of adrenal cortical hyperfunction, extensive studies were carried out. Routine blood counts and urinalysis gave normal results. The levels of serum cholesterol, calcium, inorganic phosphate, total protein, sodium and chloride were within normal limits. The CO_2 combining power was 47 and 46 vols. per cent on two occasions. A glucose tolerance curve gave values of 93 (fasting), 174 (1 hr.), 244 (2 hrs.)

and 141 mg. per cent (3 hrs.), with a corresponding drop in serum inorganic phosphate. The BMR was +1 per cent. Planographic studies of both kidneys visualized these structures satisfactorily, and no evidence of a soft tissue (*i.e.*, adrenal) mass was found. An aortic arteriogram also failed to visualize an adrenal mass.

Urinary 17-ketosteroids on two occasions were 13.0 and 13.8 mg. per twenty-four hours. Urinary gonadotropins were 3 to 5 I.U. per twenty-four hours.

Clinical course. Despite dietary measures, the administration of thyroid and amphetamine, and the most earnest protestations of cooperation, the patient continued to gain weight. She was then hospitalized and on the identical regimen lost an average of one-half pound per day. This was our first inkling of the strong psychogenic factors involved. In addition, during hospitalization urinary retention developed, on a hysterical basis according to the consulting urologist.

To regulate menstrual cyclicity, the patient was given oral estrogen and progesterone (Premarin, 3.75 mg. daily for twenty days; anhydrohydroxyprogesterone, 30 mg. daily during the last ten days) and for two cycles she responded properly to this treatment. Thereafter, despite several courses of estrogen-progesterone at greatly increased levels, menstrual flow diminished and eventually disappeared entirely. Vaginal smears showed up to 50 per cent cornification. No endometrial biopsy was possible. In view of our previous experience in Case 1, an estrogen-tolerance test was carried out, collecting urine for forty-eight hours previous to the test, administering 4 mg. of estrone in oil daily for two days, and collecting urine during these two days and for the ensuing twenty-four hours. During the control period the urinary estrogen excretion averaged 11 gamma per twenty-four hours. During three days following estrogen injection, the total excretion was 57 gamma per twenty-four hours. Thus, of the total of the 8,000 gamma injected, 24 gamma appeared in the urine—a recovery of 0.3 per cent. A vaginal smear obtained forty-eight hours after the last injection showed only 10 to 15 per cent more cornification than the pretreatment vaginal smear and still showed a considerable number of small, relatively oval-cells with large and vesicular nuclei.

DISCUSSION

Case 1 presents a number of interesting features. The early menstrual history, and particularly that of a conception, is good evidence of normal endometrial and ovarian function at that time. Normal gonadotropin values during the subsequent amenorrhea rule out the possibility of premature menopause or a major pituitary disturbance; the latter is unlikely also in view of the normal 17-ketosteroid excretion. The persistence of well developed secondary sexual characteristics in the presence of a hypoestrogenic vaginal smear, an atrophic endometrium and a low estrogen excretion is surprising but not unusual, for many postmenopausal women maintain these features almost unchanged for many years.

We had hoped that adequate priming of the endometrium with oral estrogen-progesterone would bring about the usual endometrial response in due time, but in spite of increasing and eventually massive doses, there was little vaginal bleeding and no significant change of the vaginal smear or the endometrium. It is significant that the basal body temperature curve failed to show the rise usually associated with the administra-

tion of progesterone. The response to steroid therapy was not improved by prostigmine or folic acid (given because of the hypothetical possibility of an anti-estrogen effect due to folic acid deficiency). A remarkable response of the vaginal epithelium occurred, however, when a synthetic estrogen (stilbestrol) was given.

These peculiar observations suggest two possibilities: either that the estrogen and progesterone administered were being inactivated at an unusual rate or that the target organs (endometrium, vaginal epithelium) simply failed to respond to physiologic stimulation. The possibility of the target-organ failure is unlikely for a number of reasons. It is unusual for more than one target organ to be involved, yet in this case both vaginal epithelium and endometrium failed to respond. It is also very unlikely that a target organ should first be normal (uterine pregnancy), become refractory, and then spontaneously become sensitive once more. Target-organ failure also fails to explain the low urinary estrogen excretion or the lack of temperature response to the injection of substantial doses of progesterone.

The possibility of increased steroid destruction was explored with an estrogen-tolerance test which clearly showed that the patient did destroy the injected steroid in an extraordinarily efficient manner. The failure of the injected estrogen to act on the endometrium and vaginal epithelium fortifies the conclusions of the estrogen-tolerance test. It is unfortunate that there was no opportunity to test the local effect of estrogen (by vaginal suppository) in order to confirm further the responsiveness of that tissue; however, the positive response to stilbestrol (not a steroid and therefore perhaps not inactivated by the same mechanism) is valid evidence of potential responsiveness.

It is not at all clear why an adult with low estrogen excretion levels and an apparently normal pituitary gland should not have high levels of gonadotropin excretion, although it is common knowledge that not all postmenopausal women show this elevation.

Unfortunately, it was not possible to carry out pregnanediol determinations and a "progesterone-tolerance test." The only indication available of an abnormality in progesterone metabolism was the absence of basal body temperature rise following the injection of progesterone during the period of therapy for amenorrhea, and the presence of a normal, biphasic temperature curve during the period of spontaneous cyclic menstruation in Case 1.

No data were obtained regarding the destruction or inactivation of 17-ketosteroids; however, the generally average urinary excretion would seem to indicate no profound disturbance in this sphere.

Most puzzling is the problem of the relation between the emotional

state of this patient and the return of normal spontaneous menstruation. From the vaginal smear it is evident that estrogen destruction was no longer proceeding at the extremely rapid rate observed before. Further estrogen-tolerance studies at that time were not possible, but the evidence of the vaginal smears (and also basal body temperatures) is quite clear. One can only speculate that external stresses are capable of influencing the metabolism of steroids in some manner. The mechanism of such an action is entirely obscure, although it is well known that external stresses can affect, by mediation of the pituitary, the steroid *production* of the adrenal cortex. In this case, however, we have no evidence that pituitary function, insofar as gonadotropin and adrenocorticotropin are concerned, was in any way affected; and even if such a pituitary disturbance were postulated, we have no evidence that pituitary function controls the reactions involved in the metabolism of estrogen and progesterone.

The second case, though more complex clinically and less well studied, illustrates another instance of hormone-resistant amenorrhea in which excessive destruction of injected estrogen could be shown in the absence of any demonstrable abnormality of gonadotropic activity.

Amenorrhea secondary to severe psychic trauma and with no response to administered estrogen was observed also by Paschkis and Rakoff (8) in several patients. These investigators sought without success for the presence of anti-estrogenic substances in the serum, and found (as we did) no effect from folic acid administration. Their patients, however, did not show excessive estrogen destruction, though this was studied by a different technique.

It is evident from these studies that a great deal remains to be learned about the influence and mode of action of psychic stresses on the physiology of the menstrual process, and about abnormalities such as the two types of psychogenic amenorrhea (the hormone-resistant and the more common, non-resistant variety). The "hypothalamic" hypothesis of Klinefelter *et al.* (8) and of Reifenstein (10), as one of us (J. W. G.) pointed out in a recent publication (11), is not based on satisfactory experimental evidence or even on unequivocal interpretation of the data. Hence, this problem remains one of the most unexplored in the entire field of menstrual physiology.

SUMMARY

Two cases of hormone-resistant amenorrhea are presented with clinical studies indicating a correlation with psychogenic factors. Studies of estrogen metabolism indicated in both instances an excessively rapid destruction of the administered steroid; this process was unaffected by folic acid, prostigmin or the use of extremely large doses of estrogen and progesterone.

In one case, furthermore, the absence of temperature response to progesterone suggested the possibility of abnormal progesterone metabolism as well. Spontaneous cyclic menstruation with normal vaginal smear changes and basal temperature curves reappeared during a time of emotional stability. The significance of these findings is discussed.

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ADRENAL CORTICAL TUMOR CAUSING HIRSUTISM WITHOUT OTHER EVIDENCE OF VIRILIZATION

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CASE HISTORY

A well developed 28-year-old brunette female came to the Endocrine Clinic in 1947, complaining of an extreme degree of hirsutism of fourteen years' duration. The patient's ancestry was Indian, French and Spanish. Excessive hair on the face and abdomen was first noticed at the age of 12 years, and had increased since that time. On initial examination she was found to have profuse heterosexual hypertrichosis, the beard, axillary, pubic, and body hair being much more extensive than in the average male. In addition to the hirsutism, she also displayed a degree of muscular development which was considerably more than average.

The patient's voice was feminine; her breasts were well developed; the clitoris was normal; and menstruation, which began at the age of 12 years, had always been regular.

She had had one pregnancy with a spontaneous miscarriage at five months. She also stated that her libido was somewhat more than adequate.

She was first seen in the Endocrine Clinic by Dr. L. E. Curtis, who ordered a 17-ketosteroid determination and an intravenous pyelogram. The initial 17-ketosteroid values were 82 and 62 mg. respectively (ketonic fraction) per twenty-four hours. The beta 17-ketosteroids constituted 55 per cent of the total.[1]

The intravenous pyelogram revealed displacement of the left kidney with some calcification above the upper pole. Other laboratory findings were within normal limits

Exploration of the left renal area in August, 1947, by Dr. Frederick S. Howard, and Dr. Frank Hinman, Jr. revealed a well encapsulated mass, about 4 inches in diameter, above the left kidney and continuous with the left suprarenal gland. The tumor was removed, but a small portion of the gland was permitted to remain, since it appeared normal. The anatomic relationships are shown in Figure 1.

Histologic examination of the tumor revealed some slight tendency to invasion of blood vessels. Consequently, the patient received 3,090 roentgen units of x-irradiation over the region of the left suprarenal gland (calculated at the level of the gland).

Two weeks after operation, the 17-ketosteroid excretion[2] was 6.3 mg.; two months later it was 10.2 mg.; and in April, 1948, the value reported was 19.3 mg. per twenty-four hours. (At this time the patient received the x-ray therapy.) Subsequent values for

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[1] Acknowledgment is made to Dr. Ralph I. Dorfman of Western Reserve University, for carrying out this analysis.

[2] Acknowledgment is made to the Ciba Pharmaceutical Products, Inc., and to the Schering Corporation for grants which were used in part to defray expenses of 17-ketosteroid determination.

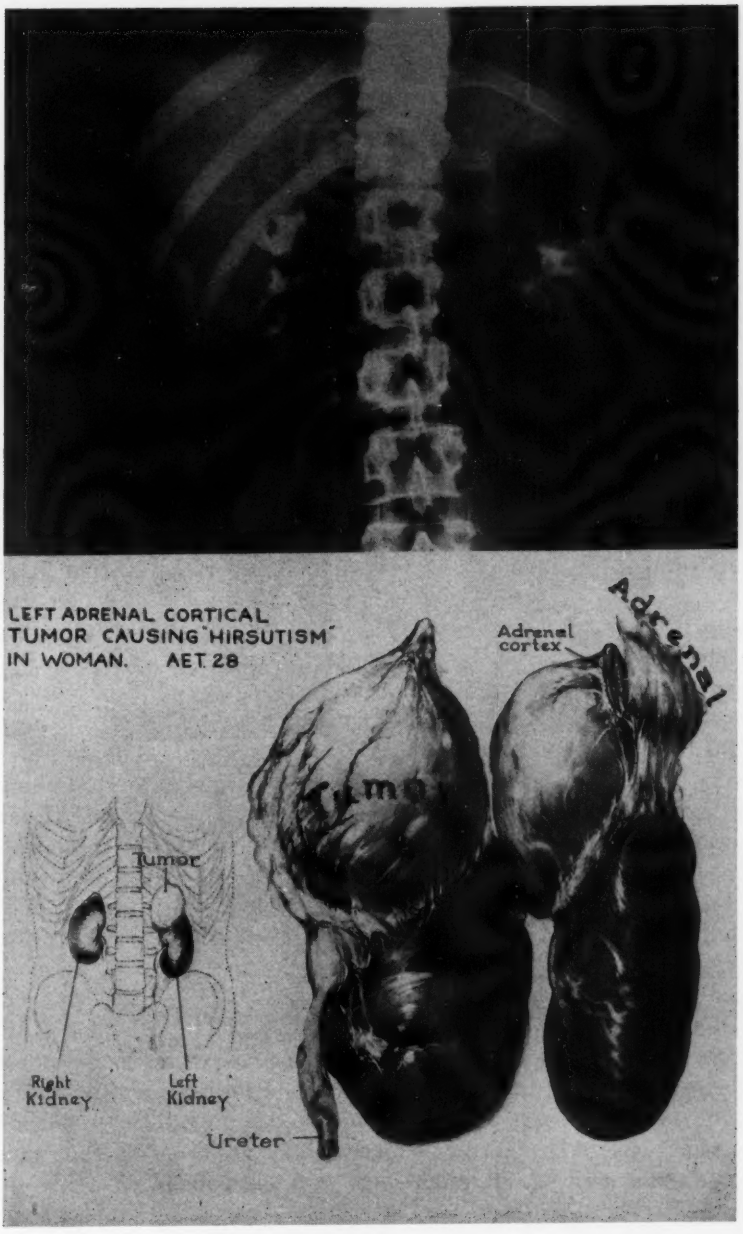
LEFT ADRENAL CORTICAL
TUMOR CAUSING "HIRSUTISM"
IN WOMAN. AET. 28
Adrenal cortex
Adrenal
Tumor
Tumor
Right Kidney
Left Kidney
Ureter

FIGURE 1

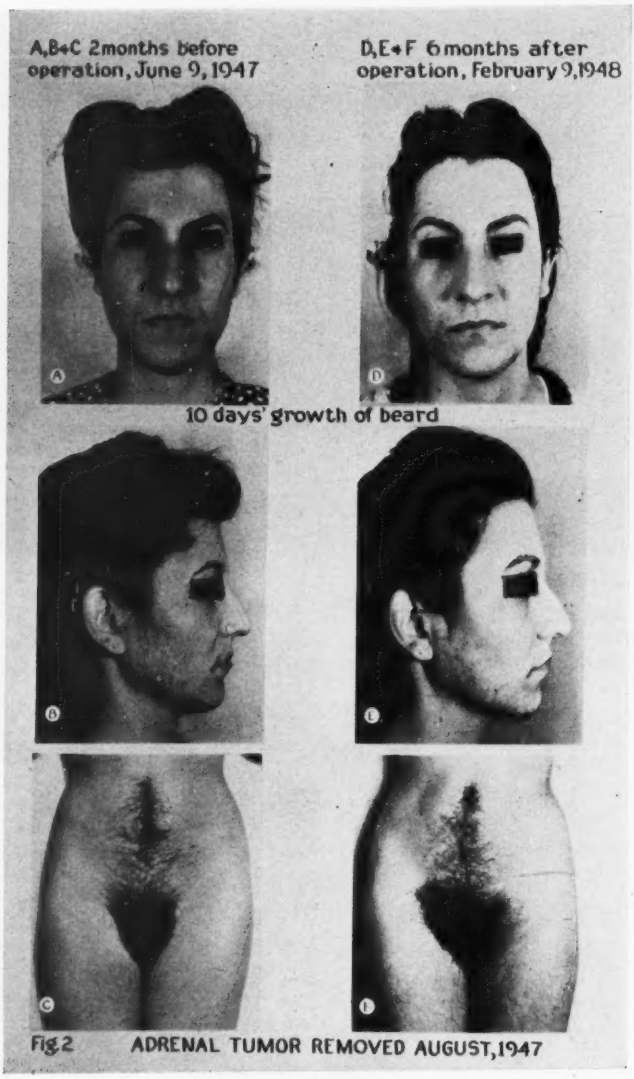
A,B+C 2 months before operation, June 9, 1947
D,E+F 6 months after operation, February 9, 1948
10 days' growth of beard
Fig 2 ADRENAL TUMOR REMOVED AUGUST, 1947

FIGURE 2

urinary 17-ketosteroids over a three-year period have remained consistently between 10 and 14 mg. per twenty-four hours.

Since removal of the tumor, the patient's clinical status has been completely normal except that a considerable degree of hirsutism remains. In Figure 2 are shown photographs of the patient before operation, and six months after operation. The ten-day growth of beard in each instance suggests that there has been a slight decrease in the rate of growth following surgery. At the present time, however, (three years after operation) it is still necessary for the patient to remove facial hair three times weekly.

DISCUSSION

There is little question that the adrenal cortex normally secretes a hormone which has a major growth-promoting (anabolic) effect, but a relatively mild androgenic (masculinizing) effect. This hormone is not secreted until the time of puberty. In the female, it is entirely responsible for the growth of axillary and pubic hair, and for the urinary 17-ketosteroids. In support of these statements are the following observations:

1. The normal female has no growth of axillary or pubic hair, nor any excretion of 17-ketosteroids until the time of puberty.

2. The female patient with Addison's disease, even though she may have perfectly normal ovarian function, excretes no urinary 17-ketosteroids, and has no growth of axillary and pubic hair.

The majority of patients with virilizing syndromes, referable to adrenal tumor or hyperplasia, have in addition to hirsutism, significant deepening of the voice, enlargement of the clitoris, and inhibition of normal ovarian function, *i.e.*, a picture such as one may produce by administration of adequate amounts of testosterone. It appears probable, therefore, that such a syndrome is referable to excessive production of an *abnormal* adrenal steroid. In the patient here reported, it seems likely that the adrenal tumor was secreting an excessive amount of the anabolic steroid which is normally manufactured by the postpubertal adrenal cortex.

While working with Dr. Fuller Albright, one of the authors had the opportunity to follow another patient with a malignant tumor of the adrenal cortex, who immediately prior to her death excreted more than a gram a day of urinary 17-ketosteroids. This patient also had abundant growth of sexual hair, continued to have normal ovarian activity, but had no deepening of the voice or enlargement of the clitoris. Of interest also is the fact that her nutrition and general muscular status remained excellent until a few weeks before her death, presumably due to the very potent anabolic effect of the steroid which was being produced by the tumor, and despite extensive metastases from the tumor to the liver and to other viscera.

Based on the foregoing observations, the value in clinical diagnosis of the urinary 17-ketosteroid determination (with all of its imperfections)

is stressed. Without the finding of high urinary 17-ketosteroids, it is probable that in view of the long duration of the hirsutism in this patient, one would have assumed that a familial variety of the condition existed. It is necessary to emphasize, however, that unfortunately not all patients with adrenal tumor or hyperplasia do have an increase in urinary 17-ketosteroids. It will be recalled that whereas free testosterone and testosterone propionate are excreted as urinary 17-ketosteroids, methyltestosterone, an equally potent androgen, is not excreted in this form.

SUMMARY

A woman with a large adrenal cortical tumor presented a clinical picture of fourteen years' duration in which the only significant deviations from normal were those of heterosexual hypertrichosis and a tendency to increased skeletal musculature. This patient emphasizes the necessity of regarding every female with hirsutism as possibly having an adrenal cortical or ovarian tumor, until it has been proven otherwise.

THE DETERMINATION OF URINARY STEROIDS. I. THE PREPARATION OF PIGMENT-FREE EXTRACTS AND A SIMPLIFIED PROCEDURE FOR THE ESTIMATION OF TOTAL 17-KETOSTEROIDS

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THE quantitative estimation of urinary 17-ketosteroids has been proven of value as an aid to the clinical diagnosis of disease. Its more widespread use has been hampered by the actual and assumed difficulties inherent in the chemical methods employed. In 1947, there was proposed a simple and rapid method (1), some disadvantages of which became apparent following its use in thousands of determinations. For instance, the extraction with ether is a dangerous hazard. Adequate control throughout the various manipulative steps is difficult, since mechanical equipment cannot be readily adapted to separatory funnels. The funnels are clumsy to handle and the breakage rate is high. When large numbers of determinations have to be made, a special laboratory is a prerequisite. In view of the solubility of ether in water and water in ether, a correction factor must be used for the final aliquot. Unless special precautions are taken to reduce evaporation, considerable error may result. Over and above these problems, there still remains the controversial point concerning the proper way to correct for interfering pigments commonly present in urine.

A solvent has been sought which will extract all the steroids with a minimal amount of impurity. Hydrolyzed urine was extracted at a rate of 5 parts of solvent to 1 part of urine, with the following substances (Table 1): ethylene dichloride, carbon tetrachloride, chloroform, methylene dichloride, isopropyl ether, ethyl ether, benzene, and petroleum ether. A 10 per cent sodium hydroxide solution was employed to remove the acidic and phenolic constituents. Results given in Table 1 show that, with the exception of petroleum ether, all the solvents gave similar values for 17-ketosteroids after correction for the urine pigment blank. With none of the solvents could the urine blank be considered a negligible read-

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TABLE 1. VALUES FOR URINARY 17-KETOSTEROIDS AND URINE BLANKS OBTAINED WITH VARIOUS SOLVENTS

Specimen	Solvent															
	$EtCl_2$		CCl_4		$CHCl_3$		CH_2Cl_2		I.P. Ether		Et. Ether		C_6H_6		Pet. Ether	
	A*	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Pooled normal ♂	15.6†	1.4	15.3	0.8	15.7	1.2	15.5	1.3	15.0	0.9	14.9	0.9	15.1	0.9	10.6	0.7
Pooled normal ♀	9.7	1.3	9.3	0.5	9.1	0.6	9.0	0.7	9.0	0.4	9.1	0.6	9.4	0.7	5.2	0.3
Pooled pathologic ♂	9.5	0.7	9.6	0.4	8.9	0.5	9.0	0.4	9.1	0.5	9.4	0.3	9.3	0.5	6.2	0.1
Pooled pathologic ♀	8.1	2.2	8.0	1.6	8.1	1.3	7.5	1.0	7.9	1.5	7.9	1.4	7.9	1.5	5.3	0.9

* A—Zimmermann value corrected for its urine blank.
B—Urine blank.
† All figures represent milligrams of dehydroisoandrosterone equivalents per liter.

ing. None of the solvents enabled us to differentiate the 17-ketosteroids from interfering pigments directly. However, ethylene dichloride was chosen, as it possessed the most desirable physical properties. These were low volatility, insolubility in water and vice versa, adaptability to mechanical equipment, convenient low temperature of evaporation, low toxicity, noninflammability, and nonexplosiveness. That ethylene dichloride removed more pigment than any other of the tested solvents was of secondary importance, since it had been decided that the only answer to the problem of the pigments was their complete elimination. Studies were made of existing methods for removing the pigments entirely from the ethylene dichloride solution. A method (2) based on purification after carrying out the Zimmermann procedure was not too practical, since it became quite involved and introduced several sources of error. Chromatographic adsorption and subsequent elution (3) of the steroids likewise had disadvantages. Furthermore not all pigments were removed. Decolorizing agents such as noxite may lead to loss of desired steroids (4). Formation of the Girard derivative for the separation of the ketonic from the nonketonic steroids eliminates a major part of the pigment but this also complicates the procedure. In addition, Dobriner and his associates (5) have pointed out that recovery of the ketosteroids after hydrolysis of the hydrazone can be a source of error. They also point out that the formation of the hydrazone is not a strictly quantitative matter. None of these procedures offered a satisfactory solution to our problem, so that a different approach became necessary.

It is well known that most of the pigments present in acid-hydrolyzed urine are formed during the hydrolysis. The refractory portion of these pigments, which remains even after exhaustive washing with aqueous sodium hydroxide, is the basis of the urinary pigment blank. The present study is concerned with a method for the removal of these pigments.

TABLE 2. RESULTS OBTAINED AFTER TREATING ETHYLENE DICHLORIDE SOLUTIONS OF STEROIDS WITH PELLETS OF SODIUM HYDROXIDE

Compound*	Source	Colorimeter readings (E×1,000) for the			
		Zimmermann reaction		Pettenkoffer reaction	
		before pellets	after pellets	before pellets	after pellets
Androsterone	Sloan-Kettering	456	444	30	22
Androsterone-3α-ol-17-one	Organon	577	570	40	40
Isoandrosterone	Schering	536	550	82	82
Androstane-3β-ol-17-one	Organon	481	487	51	54
Ethiocholane-3α-ol-17-one	Sloan-Kettering	501	505	8	10
Ethiocholane-3β-ol-17-one	Schering	496	495	10	11
Dehydroisoandrosterone	Schering	445	432	712	688
Dehydroisoandrosterone	Sloan-Kettering	498	507	700	680
Dehydroisoandrosterone acetate	Schering	420	420	640	642
Androstanedione-3,17	Schering	626	608	4	8
Δ^4 Androstenedione-3,17	Schering	635	630	18	8
Estrone	Schering	552	10	48	0
Estradiol	Schering	28	30	90	10
Pregnanediol-3α, 20α	Ayerst, McKenna & Harrison	12	23	80	80
Allo-Pregnanediol-3α, 20α	Parke, Davis & Co.	10	20	85	80
Allo-Pregnanedione-3,20	Parke, Davis & Co.	216	234	2	3
Δ^5 Pregnene-3β-ol-20-one	Schering	120	122	494	490
Δ^5 Pregnene-3β-ol-20-one	Sloan-Kettering	78	84	430	428
Δ^5, 16 Pregnediene-3β-ol-20-one	Parke, Davis & Co.	125	125	320	330
Testosterone	Parke, Davis & Co.	238	242	10	10
Progesterone	Parke, Davis & Co.	304	309	6	8

* These materials were obtained through the following individuals whose courtesy is herewith gratefully acknowledged: John R. Jewel of Ayerst, McKenna & Harrison; David McGinty of Parke, Davis & Co.; Kenneth Thompson of Organon; Konrad Dobriner and Seymour Lieberman of Sloan-Kettering; and E. L. Henderson of Schering.

Note: All compounds were assayed on an aliquot containing approximately 20 micrograms of material.

That these refractory pigments do not behave identically with the other formed pigments when treated with aqueous sodium hydroxide does not necessarily signify the lack of a potentially reactive group or groups within them. There are several possible explanations:

(a) The reactive group (or groups) of these pigments is not sufficiently

acidic under usual conditions to form a salt. Treatment with stronger alkali might prove efficacious.

(b) The salts of the refractory pigments may be soluble in the solvent saturated with water. Dehydration of the solvent might then be the answer.

(c) The presence of water may permit the dissociation of the salts of the refractory pigments, the dissociated product being soluble in ethylene dichloride. Elimination of the water phase might then be the solution.

If we assume the presence of a reactive group in the pigments, then a reagent which will incorporate the above suggestions should be effective in completely removing the pigments. Solid sodium hydroxide could be such a reagent. It was first felt that with the small amount of water carried over in the solvent after extraction of the acid-hydrolyzed urine, there should form a very strong alkaline solution which should remove not only pigments, but phenolic and acidic constituents. The excess alkali would act as an effective dehydrating agent. This theory was tested, using the colored phenol, 2, 6-dichloro-benzenone indophenol which was found to be removable from even dry ethylene dichloride. This showed that a reaction could occur between a dissolved compound and a solid, insoluble in the medium. An objection to the use of solid sodium hydroxide might be the possibility of the adsorption of neutral 17-ketosteroids on the surface of the pellets. Twenty-one steroids were tested by shaking for fifteen minutes in the presence of pellets of sodium hydroxide. Table 2 lists the results and it can be seen that there is no significant diminution or augmentation, except for the phenolic steroids as tested with the Zimmermann and Pettenkoffer reactions. Insofar as the tested steroids were concerned the the method was suitable.

METHODS

Pooled specimens of both normal and pathologic urines of both sexes were used throughout these studies. Hydrolysis, extractions, and washings were carried out in Pyrex bottles with flat-top ground-glass stoppers. Shaking was done in a modified Kahn shaker adapted to hold bottles. The shaking rate was 152 cycles per minute. Except where noted, fifteen minutes was the usual time for each shaking operation. When emulsions were encountered, centrifugation was employed. When solid alkali was employed, it was in the form of sodium hydroxide pellets C. P. from various manufacturers. The pellets were used as received and the only precaution was to keep the bottle well stoppered to prevent the adsorption of moisture.

For the Zimmermann reaction we have found it advantageous to follow the procedure of Pearson and Giaccone (6), using 8N potassium hydroxide for twenty-five minutes at 25°C. Hansen and his associates (7) have

pointed out that the use of so high a concentration of alkali yields a high blank in the Zimmermann reaction. This has been avoided in the procedure of Pearson and Giaccone. In checking this procedure against that of Holtorff and Koch (8) we have found results to be comparable in the handling of both pure hormones and urinary extracts. The time-saving feature, increased accuracy, and greater stability of color favored adoption of this modification. The values for the pigments were obtained by running a urine blank along with the Zimmermann reaction, omitting the meta-dinitrobenzene and substituting an equivalent amount of absolute alcohol.

The Pettenkoffer procedure was carried out according to the method of Hansen and his associates (9).

The Girard procedure was essentially a modification of that used by Barnett and his associates (10), except that ethylene dichloride was used as the solvent for the nonketonic fraction.

EXPERIMENTAL

The minimal effective ratio of ethylene dichloride to hydrolyzed urine was determined to be ½ to 1. However, troublesome emulsions were sometimes encountered at this ratio. Therefore, a ratio of 1 to 1 was fixed as standard procedure, to permit the accurate extraction of much larger amounts of steroid than are ordinarily encountered. Emulsions formed at this ratio were easily broken by centrifuging. The completeness of the extraction was checked by re-extracting the urine with ether. No additional material capable of producing the Zimmermann reaction was found.

A check was made on the loss of ethylene dichloride during the various manipulative steps. The loss was found to be negligible; thus the use of aliquots is justified.

The extraction time under the conditions of mechanical shaking in bottles was then studied. At a ratio of 1 part of ethylene dichloride to 1 of hydrolyzed urine, extraction was complete within five minutes. Completeness of extraction was always checked by re-extraction with ether. Since no additional effort is involved, fifteen minutes of shaking was adopted as the standard procedure.

For the purpose of removing interfering substances, including certain steroids (see Table 2) and interfering pigments, the ethylene dichloride extract was vigorously shaken with pellets of sodium hydroxide. In order to determine optimal conditions for this removal, a varied number of pellets and several different periods of shaking were tried. The results are shown in Table 3, in which values for the urine blanks and the extract were determined simultaneously by means of the Zimmermann reaction. The number of pellets listed is for 10 ml. of ethylene dichloride extract, equivalent to 10 ml. of urine. The amount of hormone present is expressed

TABLE 3. EFFECT OF NUMBER OF PELLETS AND TIME OF SHAKING ON THE VALUES FOR THE URINARY 17-KETOSTEROIDS, AND PIGMENT BLANKS

Specimen	Time of shaking→	3 Minutes				9 Minutes				15 Minutes				30 Minutes			
	No. of pellets*→	2	10	20	40	2	10	20	40	2	10	20	40	2	10	20	40
Normal ♂ urine	Zimmermann value†	15.3‡	14.7	14.0	13.6	14.7	14.2	13.6	13.4	14.5	14.1	13.6	13.1	14.0	13.7	12.6	13.5
	Urine blank	1.2	0.7	0.5	0.4	0.7	0.6	0.3	0.3	0.5	0.2	0.2	0.2	0.5	0.2	0.2	0.2
Normal ♀ urine	Zimmermann value	9.9	9.5	9.5	9.1	9.5	8.9	8.8	8.5	9.2	8.8	8.7	8.6	9.1	8.8	8.6	8.6
	Urine blank	1.5	0.9	0.8	0.7	0.9	0.7	0.5	0.2	0.8	0.6	0.3	0.2	0.8	0.5	0.3	0.2
Pathologic ♂ urine	Zimmermann value	10.1	9.8	9.1	8.7	9.7	9.5	9.1	8.7	9.0	8.8	8.6	8.7	8.8	8.6	8.7	8.6
	Urine blank	0.5	0.3	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0
Pathologic ♀ urine	Zimmermann value	8.5	8.4	8.3	7.6	8.1	7.8	7.4	7.5	7.9	7.6	7.5	7.4	7.6	7.4	7.5	7.4
	Urine blank	2.2	1.5	0.7	0.5	1.4	0.6	0.2	1.0	1.1	0.3	0.0	0.0	0.9	0.3	0.0	0.0

* Number of pellets is for 10 cc. of ethylene dichloride.

† Zimmermann values are corrected for their respective urine blanks.

‡ All figures represent milligrams of dehydroisoandrosterone equivalents per liter.

in terms of milligram equivalents of dehydroisoandrosterone per liter. It is seen that in the cases included in this study the figure for the pigment blank can be reduced to a negligible quantity; and a constant value for the Zimmermann reaction is obtained by the proper choice of pellets and shaking time.

In Table 4 is shown a comparison of the effects of pellets of sodium hydroxide and aqueous sodium hydroxide upon:

(a) the Zimmermann reaction—on the ethylene dichloride extract of urine, corrected for its urine blanks;

(b) the urine blank for (a);

(c) the Zimmermann reaction—on the nonketonic fraction separated by Girard's reagent from the ethylene dichloride extract, corrected for its urine blank;

(d) the urine blank for (c);

(e) the corrected value for ketonic steroids obtained by subtracting item (c) from item (a);

(f) the Pettenkoffer reaction corrected for its urine blank;

(g) the urine pigment blank for (f);

(h) the Pettenkoffer reaction—on the nonketonic fraction, corrected for its urine blank;

(i) the urine blank for (h);

(j) the correction for dehydroandrosterone equivalents obtained by subtracting item (h) from item (f).

All results are expressed as milligram equivalents of dehydroisoandrosterone per liter. For each 10 cc. of ethylene dichloride, 20 pellets were used and the mixture was shaken for fifteen minutes. Ten per cent sodium hy-

TABLE 4. COMPARISON OF THE EFFECTS OF PELLETS AND AQUEOUS SOLUTION OF SODIUM HYDROXIDE ON VARIOUS URINARY CONSTITUENTS

Specimen	Treatment	Milligram equivalents of dehydroisoandrosterone obtained by									
		(a) (b) Zimmermann reaction*		(c) (d) Zimmermann reaction on the nonketonic fraction†		(e) Correction for 17-KS	(f) (g) Pettenkoffer reaction		(h) (i) Pettenkoffer reaction on the nonketonic fraction		(j) Correction for DHA
		minus urine blank	urine blank	minus urine blank	urine blank		minus urine blank	urine blank	minus urine blank	urine blank	
Pooled normal ♂	NaOH pellets	13.3	0.2	0.7	0.1	12.6	5.6	0.5	3.3	0.2	2.3
Pooled normal ♂	10% aqueous NaOH	15.4	1.2	2.7	0.8	12.7	7.8	2.1	4.7	1.0	3.1
Pooled normal ♀	NaOH pellets	8.5	0.2	0.6	0.1	7.9	2.5	0.5	2.0	0.0	0.5
Pooled normal ♀	10% aqueous NaOH	9.7	1.3	1.6	0.6	8.1	4.1	1.8	2.4	0.9	1.7
Pooled path. ♂	NaOH pellets	8.7	0.0	0.9	0.0	7.8	3.5	0.6	2.7	0.3	0.8
Pooled path. ♂	10% aqueous NaOH	9.5	0.6	2.1	0.2	7.4	4.6	1.3	4.0	0.6	0.6
Pooled path. ♀	NaOH pellets	7.4	0.0	0.5	0.0	6.9	1.8	0.3	1.6	0.2	0.2
Pooled path. ♀	10% aqueous NaOH	8.2	2.2	1.5	1.4	6.7	2.3	2.2	2.0	1.1	0.3

* As performed on the ethylene dichloride extract.

† As performed on the nonketonic fraction separated from the ethylene dichloride extract by means of Girard's reagent.

droxide was employed for the aqueous alkaline wash, followed by a wash with water. The superiority of pellets over aqueous alkali in removing the pigment is evident. When pellets are used prior to the Girard separation, results are lower than with aqueous alkali. However, after obtaining the ketonic values shown in column (c) of Table 4, the results are practically identical. The blanks in the Pettenkoffer reaction are also considerably reduced. In addition the corrected values for dehydroisoandrosterone shown in column (j) are lower than when aqueous alkali is employed.

A comparison was made of this procedure with one previously published (1). Urines from normal males and females and diverse pathologic urines from both sexes were compared in 100 cases. In no case in which pellets were used did the value for the urine blank exceed 4 per cent of the total value for a 24-hour excretion; the average being less than 1 per cent.

In general, with the use of pellets the Zimmermann values ran from 5 per cent to 20 per cent lower than with the use of aqueous sodium hydroxide. Greater differences occurred in those cases in which the urine blank by the old method was excessively high. The urines of 35 of these cases showing a divergence greater than 10 per cent were subjected to the

Girard separation and the corrected value for ketonic steroids was obtained. For the ketonic fraction the correlation between the two methods was 98.2 per cent.

RECOMMENDED METHOD FOR ROUTINE DETERMINATION OF 17-KETOSTEROIDS IN URINE

A. Materials and equipment used

1. Pyrex bottles of 30-ml. and 20-ml. capacity, with flat-top ground-glass stoppers. (These bottles are solvent-tight when the stoppers are pushed in tightly with a twisting motion.)
2. Kahn box-type mechanical shaker. (Sponge rubber, 1 inch thick, is fastened to the contact surfaces used to hold the bottles in a horizontal position.)
3. Colorimeter. A filter-type photoelectric colorimeter equipped with a green 525 mμ filter and a cell capacity of 1.0 ml.
4. Concentrated hydrochloric acid, C. P.
5. Ethylene dichloride, purified.
6. Sodium hydroxide pellets, C. P.[1]
7. Meta dinitrobenzene, purified according to method of Drekter *et al.* (1). Make up a 1 per cent solution in absolute alcohol and store in refrigerator.
8. Potassium hydroxide, 8N, checked by titration against standard acid.
9. Absolute ethyl alcohol, free from interfering substances which may produce a false positive Zimmermann reaction. (We have found that the alcohol from some manufacturers causes a high blank. We suggest that each shipment of alcohol be tested before use.)
10. Dehydroisoandrosterone. A standard solution of 50 milligrams per liter in absolute alcohol.

B. Method

Pipette 10 ml. of urine into a 30-ml. Pyrex bottle. Add 3 ml. of concentrated hydrochloric acid and place in a water bath at 100° C. (With each batch of determinations set up a method blank by substituting 10 ml. of water for urine and following through with the entire procedure.) Remove from water bath after ten minutes and cool. Add 10 ml. of ethylene dichloride. Stopper and shake for fifteen minutes. Centrifuge and aspirate the top aqueous layer. Pour through #1 filter paper into a 20-ml. bottle. Add approximately 20 pellets of sodium hydroxide, stopper and shake for fifteen minutes. Filter through #1 filter paper. Pipette 2 ml. of the ethylene dichloride solution into a test tube and dry in a water bath at 100° C. No trace of solvent should remain. When cool, add 0.4 ml. of 1 per cent metadinitrobenzene. Rotate the tube to dissolve the dried material. Add 0.3 ml. of 8 N potassium hydroxide. Mix and place in a water bath at 25° C. Dry 0.4 ml. of the standard in a test tube and add the Zimmermann reagents as above. Similarly a Zimmermann reagent blank is prepared. After exactly twenty-five minutes add 2 ml. of 75 per cent alcohol. Mix well. Set the Zimmermann reagent blank at 100 per cent transmission. Read the standard. Next reset the method blank at 100 per cent transmission. Read the unknowns. The standard is equivalent to 10 mg./liter of 17-ketosteroids expressed as dehydroisoandro-

[1] We use Fisher Scientific Company's "electrolytic-pellets"—about 15 to 20 per determination. These pellets weigh approximately 100 milligrams each.

sterone and the calculation of the unknowns is based upon the recommendation of the manufacturer of the colorimeter employed. If the colorimeter to be used has a capacity greater than 2.7 ml., then a larger aliquot of the ethylene dichloride extract can be taken, and the amount of reagents used for the Zimmermann test increased as needed.

DISCUSSION

Aside from its advantageous physical properties, ethylene dichloride can extract the steroid hormones from hydrolyzed urine at a relatively low ratio of ethylene dichloride to urine. This is of great importance for a routine laboratory method. It eliminates the need for handling and evaporating large quantities of solvent. Furthermore, it eliminates the necessity for the bulky equipment required in carrying out previously employed methods. It removes more pigment than the other solvents, but this disadvantage was overcome when a method was found for reducing the refractory pigments to a negligible amount. This method was based on the reaction which takes place between solid sodium hydroxide and the acidic, phenolic, and pigmented constituents of hydrolyzed urine, even under relatively anhydrous conditions. When a 10-ml. sample of ethylene dichloride was treated with 20 pellets of sodium hydroxide no sodium could be detected by flame photometry in the ethylene dichloride fraction (11). Since sodium can be detected in the concentration of 0.01 microgram per milliliter, it is unlikely that our method of purification of the urinary extract depends upon the solubility of sodium hydroxide in ethylene dichloride.

It is evident from Table 2 that no reaction occurs between the solid sodium hydroxide and steroids. There was neither an increase nor a decrease in the 17, 3 or 20-ketosteroids or in the nonketonic substances as measured by the Zimmermann or Pettenkoffer reactions. In these respects, the results show an analogy between the action of pellets and aqueous solutions of sodium hydroxide. However, there are certain important differences. Practically all pigments are removed by pellets. The experiments with phenols indicated that there was definite salt formation. We feel that pellets of sodium hydroxide under the conditions specified form salts with the refractory pigments, which can then be easily removed from ethylene dichloride.

This process likewise causes a considerable reduction of the nonketonic fraction. This gives an atypical Zimmermann color and its value is significantly higher than the value for the intrinsic pigment color present when aqueous alkali is employed. That this reduction is always associated with a concomitant reduction in the pigments, tends to support the suggestion made by other investigators (4) (12) that the pigments can give the Zimmermann reaction. However, the possibility of some other material behaving

in a similar manner cannot be completely eliminated by these experiments. Regardless of the cause, another advantage of the pellets over an aqueous alkali treatment is the better colors obtained with urine extracts in the final Zimmermann reaction. It must be remembered, however, that this method does not eliminate the atypical Zimmermann colors which may be given when ketonic steroids other than 17-ketosteroids are present.

Although, by the procedure described, an improvement is also obtained with the Pettenkoffer reaction, sufficient impurities remain so that only rarely does the true blue-green color appear in the original pellet-treated extract. Since a ketone group is not necessary for the Pettenkoffer reaction the large changes found after Girard separation are expected. An additional advantage of the pellet process is that the nonketonic fraction can be obtained free from pigments. This obviously facilitates the study of this fraction. If the value for the nonketonic fraction is subtracted from the total Pettenkoffer value after treatment with pellets, the result approximates those reported in the literature for dehydroisoandrosterone.

There is little doubt that the present method of employing pellets rather than aqueous sodium hydroxide has many advantages. For satisfactory clinical results, the Girard separation can now be eliminated. There is no longer a need for a urine blank or a water wash. A dry extract is obtained and any subsequent separation can be performed without further dehydration. Mechanical equipment can now be used, which affords control of the extraction and shaking times, and eliminates laborious physical effort. The steroids can be extracted in a single step in which low ratios of solvent to urine can be used advantageously. It is thus possible to perform many determinations rapidly, conveniently, and accurately. The use of a micro-colorimeter is not an absolute necessity, as the method is quite flexible and can be easily adapted to other micro or macro procedures.

SUMMARY

A simplified method for the routine determination of urinary 17-ketosteroids is presented. Ethylene dichloride is used as the extraction agent at a low ratio of solvent to hydrolyzed urine. Sodium hydroxide pellets are used to remove interfering urinary constituents. The treatment with pellets is superior to the customary treatment with aqueous sodium hydroxide, which only partially removes urinary pigments. Furthermore, there is a considerable reduction in the nonketonic fraction, which for routine clinical purposes eliminates the need for a Girard separation. It has also been noted that some improvement in the specificity of the Pettenkoffer reaction for dehydroisoandrosterone is simultaneously obtained. By the method described, dried colorless extracts are obtained

which eliminate the need for a urine blank and a water wash. Mechanical equipment is employed for extracting and shaking. The method can be readily adapted to either a micro or macro scale.

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CLINICAL METHOD FOR THE CHROMATOGRAPHIC-COLORIMETRIC DETERMINATION OF URINARY 17-KETOSTEROIDS. II. NORMAL ADULTS

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IN 1946 (1) we published in this Journal a method for the determination of individual 17-ketosteroids in the daily output of urine. Since then many hundred analyses have been made by this method, for clinical purposes. It goes without saying that during this period improvements in the method have been introduced from time to time. We, therefore, think it desirable to publish a new description of the procedure, in its definitive form. In this article we shall also discuss the results of analysis of the urine of a number of normal men and women. These figures have been worked up statistically and serve for the definition of the normal 17-ketosteroid pattern.

METHOD

1. *Extraction*

Part of the 72-hour urine sample (A)[1] (1 to 1.5 liters) is rendered neutral by addition of hydrochloric acid (if necessary); after addition of $\frac{1}{3}$ volume of benzene (C.P.) it is heated under reflux on a steam bath for five hours (B).[1] This procedure is repeated once or twice. The benzene extracts are bulked and evaporated, after which the residue is dissolved in 10 ml. of ethanol.

The residual urine is now mixed with 1/10 volume of 25 per cent HCl and again extracted two or three times (C)[1] with $\frac{1}{3}$ volume of benzene for five hours on the steam bath. These extracts are also bulked, washed and evaporated, the residue then being dissolved in 10 ml. of ethanol as before (D).[1]

2. *Chromatographic analysis*

As a rule the amount of urine extract used for this analysis corresponds to one fifth of a 24-hour specimen (E).[1] The calculated amounts of the neutral and the acid extract are pipetted into the same Erlenmeyer flask, after which the solvent is evaporated. The residue is taken up in 50 ml. of dry benzene (C.P.). The chromatography tube is filled with 15 Gm. of aluminia (Brockmann), the water content of which is in equilibrium with the vapor pressure of 58 per cent H_2SO_4 (F).[1] The column is moistened with benzene. The urine extract is poured onto the column; after it has been adsorbed, elution is carried out according to the program shown in Table 1.

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[1] See section entitled "Comments."

TABLE 1

Numbers of eluates	Elution liquid (per eluate: 50 ml.)	Eluated 17-ketosteroids
1– 3	benzene	Δ2-androsten-17-one 3-chloro-Δ5-androsten-17-one Δ3:5-androstadien-17-one
4– 5	benzene	none
6– 8	benzene	*i*-androstan-6-ol-17-one
9–10	benzene+0.1 vol. per cent ethanol	none
11–13	benzene+0.1 vol. per cent ethanol	dehydroepiandrosterone epiandrosterone
14	benzene+0.1 vol. per cent ethanol	none
15–19	benzene+0.1 vol. per cent ethanol	androsterone Δ9-androsten-3(α)-ol-17-one
20–21	benzene+0.1 vol. per cent ethanol	none
22–30	benzene+0.1 vol. per cent ethanol	etiocholan-3(α)-ol-17-one Δ9-etiocholen-3(α)-ol-17-one
31–32	benzene+0.1 vol. per cent ethanol	none
33–38	benzene+0.5 vol. per cent ethanol	? 11-hydroxyandrosterone
39–40	benzene+0.5 vol. per cent ethanol	none
41–43	benzene+0.5 vol. per cent ethanol	11-hydroxyetiocholanolone
44	benzene+2.0 vol. per cent ethanol	none
45–48	benzene+2.0 vol. per cent ethanol	unidentified 17-ketosteroids

3. *Determination of the 17-ketosteroid content of the eluates*

The 17-ketosteroid content of each eluate is determined by means of the Zimmermann reaction, with the procedure described by Callow and co-workers (2).

One tenth of each eluate (*i.e.*, 5 ml.) is placed in a 10-ml. volumetric flask. The solvent is evaporated on an electric hot-plate at a moderate temperature. The reagents are pipetted onto the dry residue. For further details of the colorimetric method and the specifications for the reagents, the publications of Callow and co-workers should be consulted.

The color densities of the colored final solutions are determined with a Klett and Summerson photoelectric colorimeter at a wave length of 520 mμ. For the eluates #1–8 and #40–48 the color density is also measured at 420 mμ (G)[1] in order that a correction may be made for atypical chromogens which are present in these eluates.

4. *Calculation of the amounts of 17-ketosteroids in the eluates*

For the eluates #1–8 and #40–48 the corrected extinction is calculated from the formula

$$E_{gk} = \frac{K_i \times E_g - E_v}{K_i - K}$$

where

E_{gk} = corrected extinction
E_g = extinction read at 520 mμ
E_v = extinction read at 420 mμ
K_i, K_s = constants (H)[1]

The amounts by weight of 17-ketosteroids in the eluates are then calculated from the extinction produced by 100 μg. of dehydroepiandrosterone (I).[1]

The result of a chromatographic-colorimetric analysis of an extract of the urine of a 19-year-old man is represented graphically in Figure 1. Each peak on the curve (which has no mathematical significance) corresponds to one or more 17-ketosteroids (J).[1]

5. Separation of saturated and unsaturated 17-ketosteroids

Some of the peaks correspond to more than one 17-ketosteroid, *viz.*, peak I (Δ3:5-androstadienone+3-chloro-Δ5-androstenone+Δ2-androstenone), III (dehydroepiandrosterone[2]+epiandrosterone[2]), IV (androsterone+Δ9-androstenolone) and V (etiocholanolone+Δ9-etiocholenolone).

Except in peak I we always find a saturated and an unsaturated 17-ketosteroid together in one eluate. Further differentiation can be achieved here by introducing—in addition to the Zimmermann reagent—a reagent that reacts in a quantitatively different

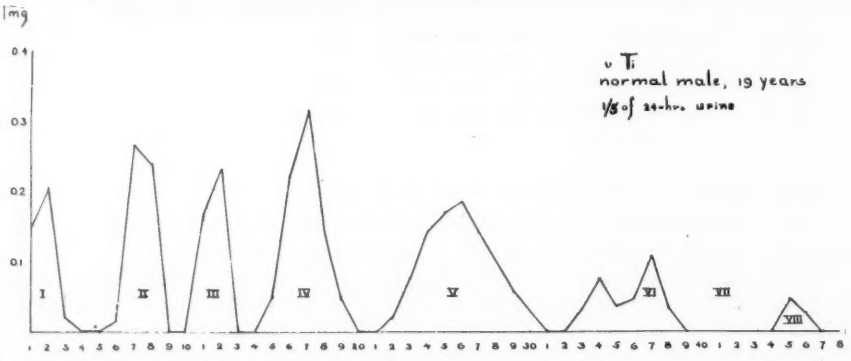


FIG. 1. (On the abscissa are shown the numbers of the eluates; on the ordinate, the amount of 17-ketosteroids in milligrams.)

Maximum
I. Δ2-androsten-17-one, 3-chloro-Δ5-androsten-17-one, Δ3:5-androstadien-17-one
II. *i*-androstan-6-ol-17-one
III. dehydroepiandrosterone, epiandrosterone
IV. androsterone, Δ9-androsten-3(α)-ol-17-one
V. etiocholan-3(α)-ol-17-one, Δ9-etiocholen-3(α)-ol-17-one
VI. 11-hydroxyandrosterone
VII. 11-hydroxyetiocholan-3(α)-ol-17-one
VIII. unidentified 17-ketosteroids

[2] Dehydroisoandrosterone+isoandrosterone

manner with saturated and unsaturated 17-ketosteroids. Pincus' reagent ($SbCl_3$ in acetic acid plus acetic anhydride 9:1) is suitable for this purpose.

Of each eluate corresponding to such a peak, 10 ml. is pipetted into an Erlenmeyer flask; the solvent is evaporated and the residue taken up in 5 ml. of ethanol. Of this solution, 2.5 ml. is pipetted into a test tube. After evaporation to dryness the residue is treated with Pincus' reagent. For details of the procedure and the specifications for the reagents, consult the report of Pincus (3).

The values obtained by means of the Pincus reaction are expressed in milligrams of androsterone.

Calculation: With the Pincus method, as carried out in our laboratory, the color of 1 mg. of dehydroepiandrosterone corresponds to that of $\frac{1}{3}$ mg. of androsterone; and that of 1 mg. of Δ9-androsten-3(α)-ol-17-one to that of $\frac{1}{2}$ mg. of androsterone (the value for Δ9-etiocholen-3(α)-ol-17-one was not determined; it is assumed that this is the same as for Δ9-androsten-3(α)-ol-17-one).

For peak III we find, thus,

$X_1 + Y_1 = Z_1$ Z_1 = Zimmermann color expressed in mg. of 17-ketosteroid
$X_1 + \frac{1}{3}Y_1 = P_1$ P_1 = Pincus color in mg. of androsterone
X_1 = mg. of epiandrosterone
Y_1 = mg. of dehydroepiandrosterone

For peak IV we find:

$X_2 + Y_2 = Z_2$ X_2 = mg. of androsterone
$X_2 + \frac{1}{2}Y_2 = P_2$ Y_2 = mg. of Δ9-androstenolone

For peak V the calculation is the same as for IV (K).[1]

For the sake of clearness and simplicity the results are shown by means of a column diagram (Fig. 2).

Figure 2 shows the same 17-ketosteroid pattern as Figure 1. Under A the diagram of Figure 1 is simply expressed in the form of columns. The height of each column gives the amount of 17-ketosteroid per twenty-four hours. Figure 2B shows the same diagram again but now after application of the Pincus reaction. The fourth and fifth columns are here somewhat lower. This is due to the fact that the androsterone and etiocholanolone fractions were found to contain small amounts of Δ9-androstenolone and Δ9-etiocholenolone, respectively. These 17-ketosteroids had been formed from 11-hydroxy-androsterone and 11-hydroxyetiocholanolone. In Figure 2B, therefore, the weight of Δ9-androstenolone detected by means of the Pincus reaction in the "androsterone" fraction is added to the 11-hydroxyandrosterone column (sixth) and the Δ9-etiocholenolone to the 11-hydroxyetiocholanolone column (seventh). Figure 2B, thus, gives the closest approximation to the original composition of the 17-ketosteroid mixture in the urine of this man.

6. *Duration of the analysis*

The method just described was designed for the analysis of a large number of urine extracts for 17-ketosteroid pattern. It was, therefore, essential that the procedure should not be lengthy or complicated, as this would have made it impossible to provide data on a large number of pathologic and normal cases within a limited time. The complete analysis required about twelve working-days of one analyst handling 14 extracts at a time.

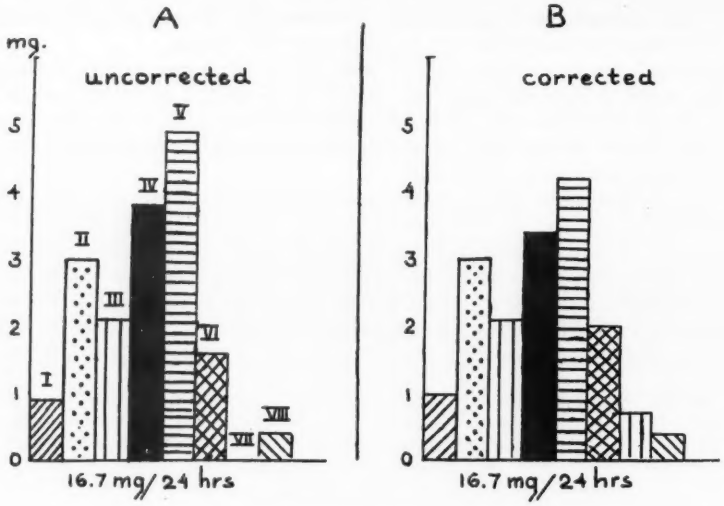


FIG. 2. I. nonalcoholic 17-ketosteroids
II. *i*-androstanolone
III. β-ketosteroids
IV. androsterone (+Δ9-androstenolone)
V. etiocholanolone (+Δ9-etiocholenolone)
VI. 11-hydroxyandrosterone
VII. 11-hydroxyetiocholanolone
VIII. unidentified 17-ketosteroids

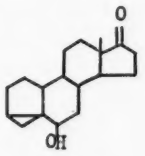
Comments

A. The use of 72-hour urine samples is intended to even up the daily fluctuations in 17-ketosteroid excretion to some extent. As can be seen from Tables 2 and 3, these fluctuations are rather large in normal test subjects. The use of a 72-hour sample is particularly advisable in those cases in which the urinary 17-ketosteroid content is determined only once.

B. The 17-ketosteroids in the urine are present chiefly in the form of esters of sulfuric acid and glucuronides. As a rule, only a relatively small percentage of the total amount of 17-ketosteroids is present in an unconjugated state.

Heating of the *neutral* urine on the steam bath under simultaneous extraction with benzene causes the hydrolysis of a certain conjugate. Urine of patients suffering from adrenal neoplasm contains a high percentage of this conjugate (up to 80 per cent of the total amount). From extracts of neutral urine we were able to isolate *i*-androstan-6-ol-17-one (4, 5, 6). The action of hydrochloric acid at room temperature converts this substance into 3-chloroandrosten-17-one, which in its turn, is con-

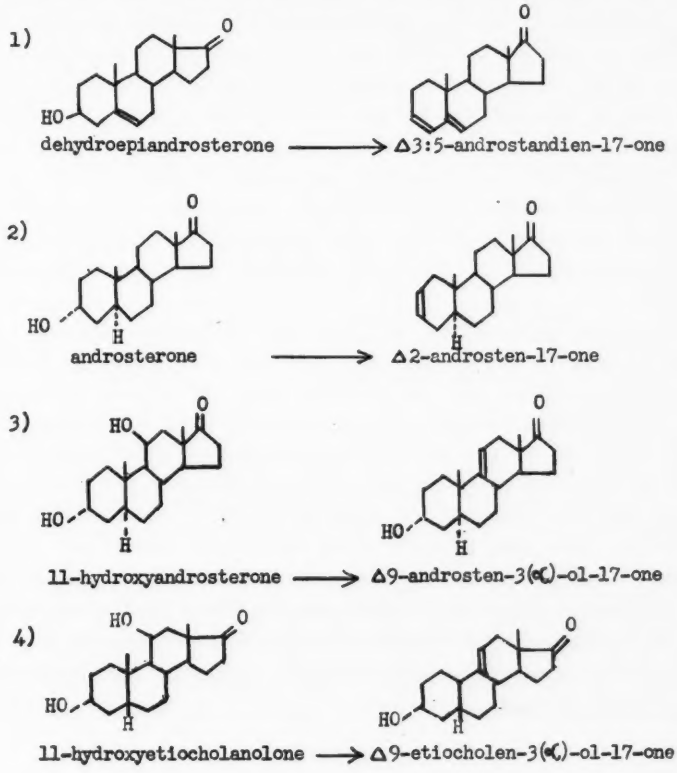
verted to a large extent into dehydroepiandrosterone on heating with acid.



i-androstan-6-ol-17-one

C. As a rule the first acid benzene extract contains 67 to 70 per cent of the weight of 17-ketosteroids still present in the urine residue. The second acid extract contains 20 to 25 per cent. Two extractions of the acid urine, thus, suffice to give a yield of 90 per cent. In many cases this is sufficient for clinical analyses.

D. The strong mineral acid required for hydrolysis of the conjugates causes dehydration of part of the 17-ketosteroids originally present. The following examples of dehydration of 17-ketosteroids by HCl are known:



This formation of artefacts complicates the final results of the analysis and must be avoided as far as possible. To prevent dehydration the hydrolysis must be done at the lowest possible temperature and with the shortest possible heating. As the hydrolysis must, on the other hand, be complete, a specially-devised method is necessary for the fulfilment of both these conditions. This consists in simultaneous hydrolysis and extraction, which ensures that the liberated steroids are removed as rapidly as possible from the sphere of action of the acid. Although this method cannot entirely prevent the formation of artefacts, it does succeed in giving extracts containing much less of these substances than extracts obtained after separate hydrolysis.

E. The total amount of 17-ketosteroids placed on a column of 15 Gm. of Al_2O_3 may not exceed 6 mg. In analysis of the urine of patients with a greatly increased excretion of 17-ketosteroids it is, therefore, necessary to work with amounts of extract corresponding to 1/10, 1/20 or sometimes even 1/100 of a 24-hour specimen.

F. The commercially-obtainable Brockmann Al_2O_3 is not entirely constant as regards its adsorptive properties. These properties are a function of the grain size, which in its turn is greatly dependent on the method of preparation and the moisture content. It was found possible to obtain Al_2O_3 with constant adsorptive properties in the following way: before use, the commercial product was always stored in a desiccator over 58 per cent H_2SO_4 until equilibrium had been established between the vapor pressure of the sulphuric acid solution and the sample of Al_2O_3 (about a week); then the concentration of the sulphuric acid solution was checked and if necessary corrected to 58 per cent. This process was repeated until the sulphuric acid solution after a week's standing showed a value of 58 ± 0.5 per cent.

G. The *crude* urine extract was used for the chromatographic analysis. Only in the case of urine samples containing large amounts of estrogens (*e.g.*, pregnancy urine) did we deviate from this rule and separate the 17-ketosteroids into neutral and phenolic fractions by means of KOH. The avoidance of further purification (girardizing, extraction with alkali or Na_2CO_3) means a great saving of time. It was found, however, that in some fractions a correction for atypical chromogens was necessary, *viz.*, in fractions #1–8 and #40–48. This correction takes very little time provided two photoelectric colorimeters are available, so that readings of the extinction at two wave lengths can be taken on the same solution. We used the method of correction for atypical chromogens as described by Fraser and co-workers (7). These authors took their idea from the work of Gibson and Evelyn.

H. K_s is $= \dfrac{\text{extinction of pure 17-ketosteroid at 420 m}\mu}{\text{extinction of pure 17-ketosteroid at 520 m}\mu}$.

This constant, thus, is easily determined by the use of a solution of a pure 17-ketosteroid. The value of K_s is about 0.4. K_i is an analogous factor for the atypical chromogens. A solution containing atypical chromogens is obtained by treatment of a urine extract with Girard's reagent. The non-ketonic fraction then serves as a solution of "pure" atypical chromogens. The value found for K_i was about 2.4.

This method of correction cannot be regarded as quite exact, since it is based on certain assumptions which are not entirely correct: *i.e.*, that the atypical chromogens are the same in every urine extract and that this mixture of compounds behaves colorimetrically as a single compound.

I. Here we made use of the observations of Callow and co-workers that equal weights of different 17-ketosteroids develop quantitatively the same color with Zimmermann's reagent.

J. The 17-ketosteroids are identified by their position in the chromatogram. The method is, thus, the same as that used for the identification of amino-acids in a partition chromatogram. The objection may, however, be raised that whereas this method is justified in the case of partition chromatography because the distribution factor is independent of the concentration of the solution, this is not true of a mixture of 17-ketosteroids on an Al_2O_3 column. It has been found, however, that in the latter case also it is possible to achieve identification with complete certainty on the basis of the position in the chromatogram, *provided the amount of adsorbent is very large in comparison with the amount of 17-ketosteroids adsorbed.* The weight of adsorbent must be at least 2,500 times that of the adsorbed 17-ketosteroids. When this condition is fulfilled it is found that a given compound always appears in the same position on the column (Fig. 3).

The effect of the large amount of adsorbent is, in the first place, that the zones formed by different 17-ketosteroids are separated by bands in which the 17-ketosteroid concentration is ~ 0. This is necessary for a satisfactory determination of the weights of the different compounds. It also means that the situation for each individual 17-ketosteroid is the same as if a simple solution of the compound in question had been placed on the column, since the different 17-ketosteroids do not influence each other during elution.

In connection with his "frontal analysis" Tiselius (see Claesson (8)) introduced the concept of retention volume. This retention volume is the amount of liquid that runs through the column before the adsorbed material is eluted. The retention volume is dependent on the concentration of the solution placed on the column: the higher the concentration (C)

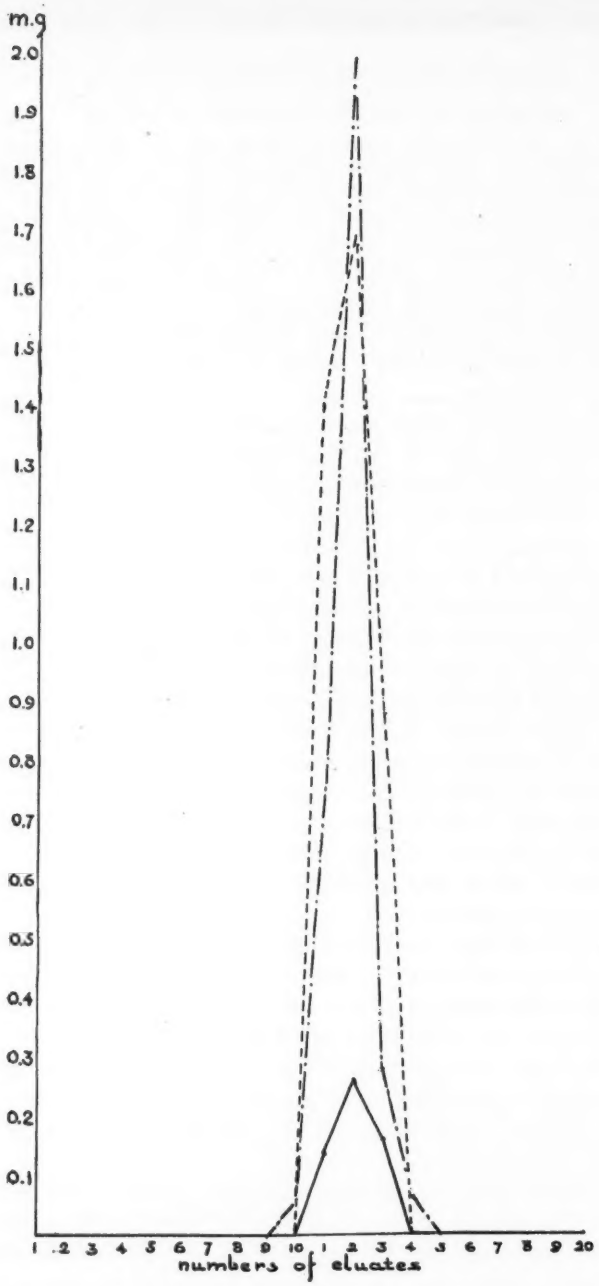


FIG. 3. —·— 4.0 mg. dehydroepiandrosterone (crystalline) + urinary extract
——— 3.0 mg. dehydroepiandrosterone (crystalline)
—— 0.5 mg. dehydroepiandrosterone (crystalline)

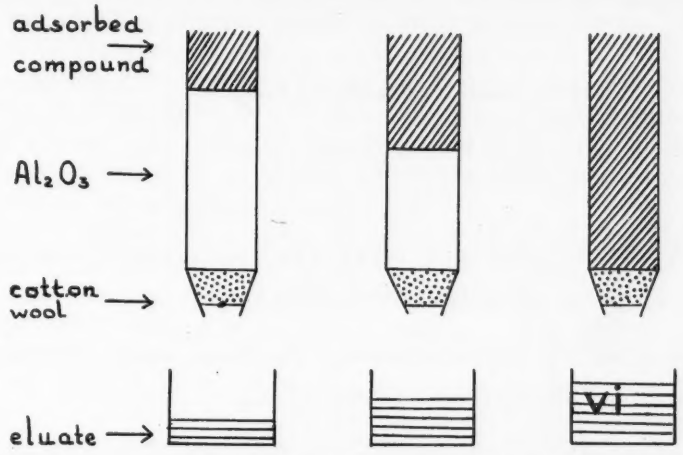


FIG. 4. Diagram of Tiselius' experiments (see text).

the lower will be the value of the retention volume (Vi). Where C is very low, however, Vi approaches a constant value.

In Tiselius' experiments, the column was rinsed through with the solution of concentration C, so that at the moment when the amount of liquid that had passed through was Vi, the whole column was filled with adsorbed material.

In our 17-ketosteroid analyses, we rinsed the column through with the pure solvent, so that just before the adsorbed material left the column

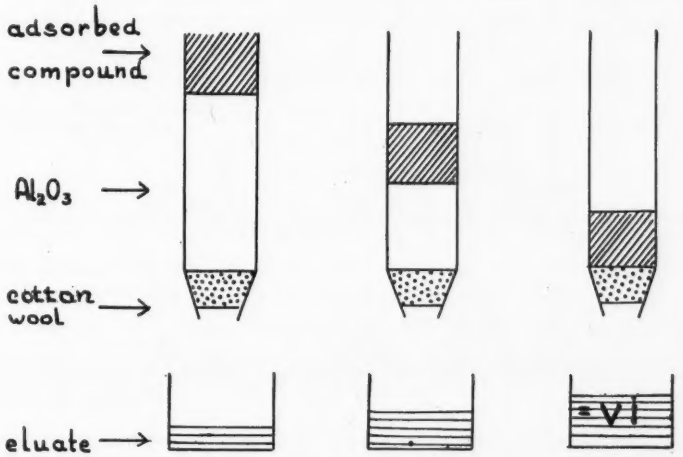


FIG. 5. Diagram of authors' method of 17-ketosteroid analysis (see text).

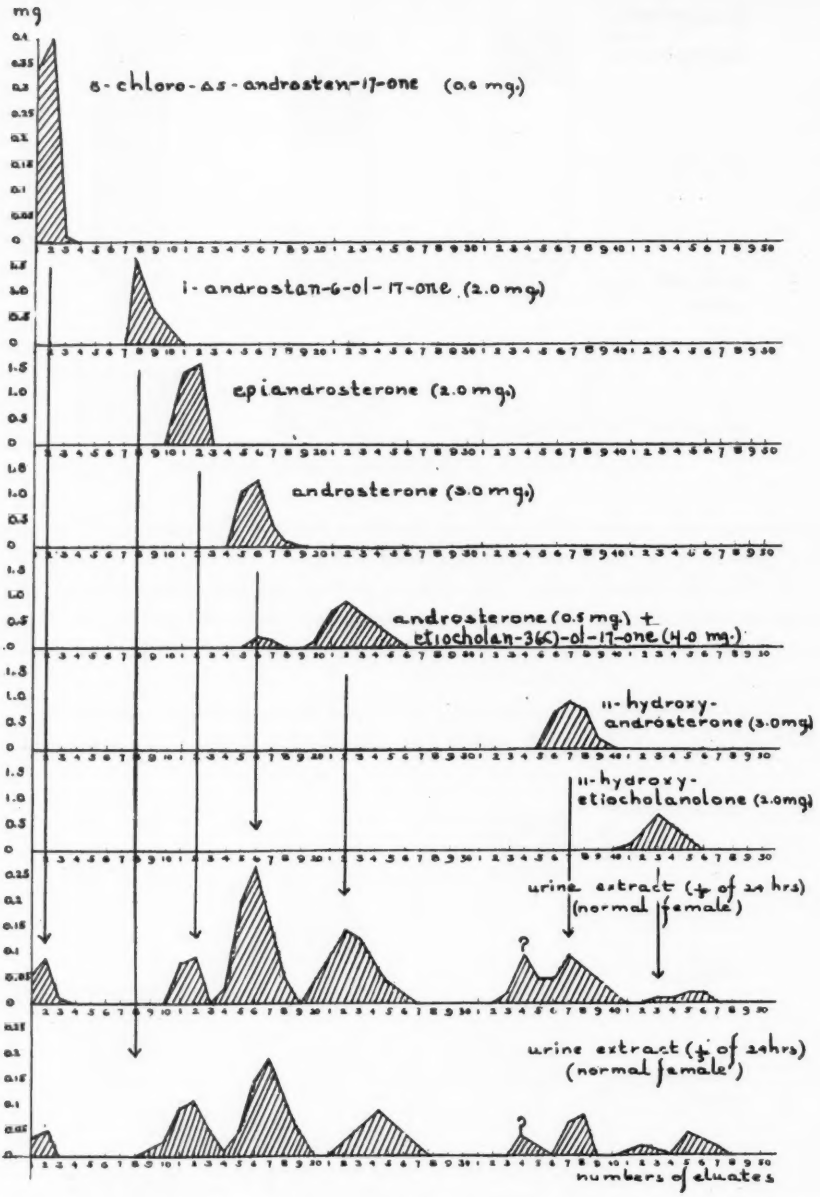

mg
6-chloro-Δ5-androsten-17-one (0.6 mg.)
1-androstan-6-ol-17-one (2.0 mg.)
epiandrosterone (2.0 mg.)
androsterone (3.0 mg.)
androsterone (0.5 mg.) + etiocholan-3(G)-ol-17-one (4.0 mg.)
11-hydroxy-androsterone (3.0 mg.)
11-hydroxy-etiocholanolone (2.0 mg.)
urine extract (¼ of 24 hrs) (normal female)
urine extract (¼ of 24 hrs) (normal female)
numbers of eluates

FIG. 6. Identification of pure compounds by chromatography. (On the abscissa are shown the numbers of the eluates; on the ordinate, the amount of 17-ketosteroids in milligrams.)

there was adsorbed material present in the lower part of the column, while above this was a region with a concentration of ~0.

The situation of the "front" molecules will, however, be exactly the same as in Tiselius' experiment. It is obvious that in our experiments, too, the "front" molecules were being continually pushed on by a very dilute solution, *i.e.*, the solution in equilibrium with the layer of adsorbed material on the column immediately above the "front."

In the chromatography of urine extracts there is also the possibility of ascertaining the position of adsorption of colorless compounds by means of the adsorption of colored compounds having the same adsorption properties. Practically all urine extracts contain a small amount of a red coloring-matter, uroroseine. This substance has, in our standard procedure, about the same adsorption properties as dehydroepiandrosterone and is, therefore, always found in the eluates #11–12. This makes it possible to determine without difficulty the place of dehydroepiandrosterone, which provides an extra guarantee of the correctness of the conclusions as to the identity of the various peaks.

The identification of these peaks was carried out in various ways:

1) By chromatography of pure compounds (as shown in Figure 6);

2) By addition of pure "urinary" 17-ketosteroids to urine extracts of known composition. For instance, if dehydroepiandrosterone or epiandrosterone is added, the peak III is raised. (The mean recovery of added substance is 92 per cent.) (See also Dingemanse and co-workers (1).)

3) After all the known "urinary" 17-ketosteroids had been added, there remained a few unidentified maxima (II, VII and VIII). Larger amounts of these three substances were collected and subjected to further purification until crystalline products were obtained. (For the identification of maximum II, see Dingemanse and co-workers (4, 5).)

Maximum VII was found to correspond to a 17-ketosteroid of m.p. 237–238° C. Its acetate had m.p. 238–238.5° C. This shows that maximum VII corresponds to 11-hydroxy-etiocholan-3(α)-ol-17-one.

K. The proofs of the correctness of this calculation are to be found in the observations recorded in Figure 7. Mixtures of dehydroepiandrosterone and androsterone[3] and of androsterone and Δ9-androsten-3(α)-ol-17-one were subjected to the Pincus reaction. It was found that the extinctions of the individual components were represented additively.

We did not use the Pincus reaction for all our extracts as we did not realize at the beginning of the investigation that more than one ketosteroid was present in maxima IV and V. Quite soon, however, it was noted that

[3] Epiandrosterone was not available insufficient quantity. This compound and androsterone, however, show identical behavior with respect to the Pincus reagent.

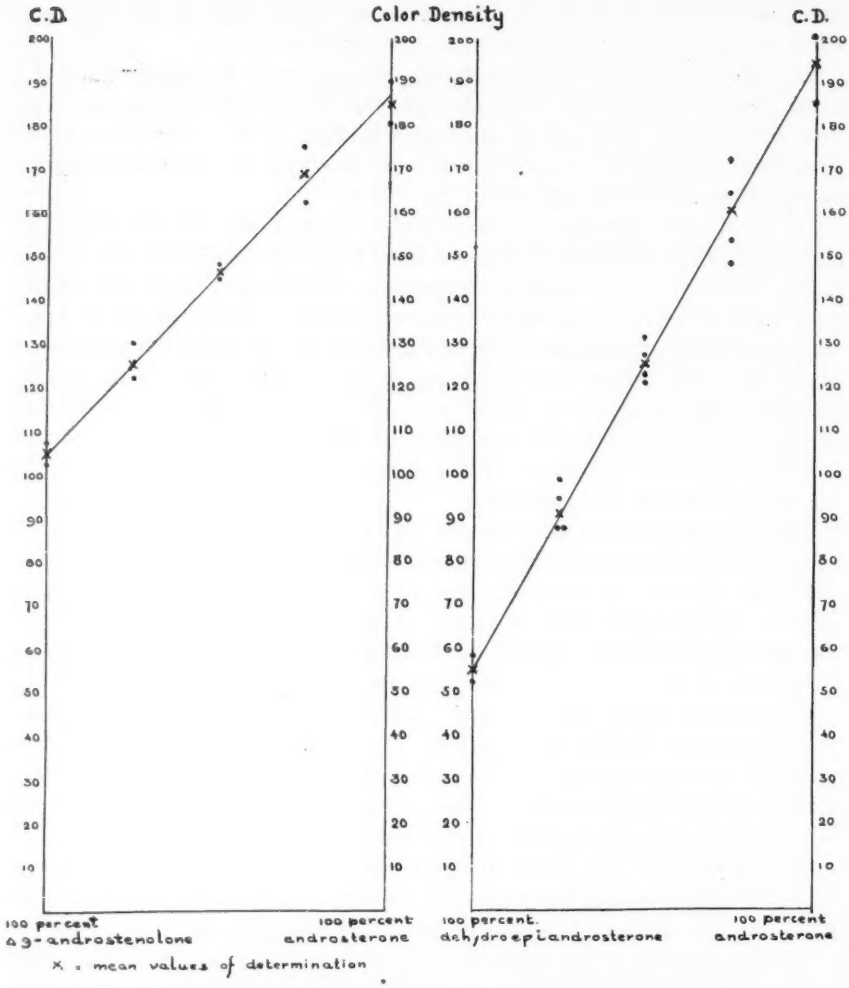


FIG. 7. The Pincus reaction applied to mixtures of 17-ketosteroids.

the biologic activity on the capon comb, of certain "androsterone fractions" from patients with hyperplasia of the adrenal cortex was much lower than was to be expected from the colorimetric readings. Publications of American authors (Liebermann *et al.* (9)) provided the explanation. The compound Δ9-androsten-3(α)-ol-17-one which is eluted together with androsterone, has only $\frac{1}{3}$ of the biologic activity of the latter, but a high content of this dehydration product of 11-hydroxyandrosterone is to be expected in the urine of patients with adrenal hyperplasia.

TABLE 2. NORMAL WOMEN

Age	17-Ketosteroids in mg./24 hrs.							
	I Non-alcoholic 17-keto-steroids	II *i*-Andro-stanol-6-one-17	III *β*-Keto-steroids	IV Andro-sterone (+Δ9-andro-stenolone)	V Etiocholan-olone (+Δ9-etiocholen-olone)	VI+VII 11-Hy-droxy-17-keto-steroids	VIII Uniden-tified fraction	Total
18	0.5	0.0	1.5	2.6	1.5	1.3	0.3	7.7
20	0.8	0.0	0.9	3.6	2.5	2.2	0.4	10.4
21	1.1	1.0	1.8	3.5	3.3	1.9	0.3	12.9
26	0.3	0.1	1.3	4.9	4.3	1.9	0.1	12.9
29	2.5	0.2	1.3	4.0	5.0	1.3	0.8	15.1
29	2.5	0.5	3.3	4.0	5.2	1.5	1.0	18.0
29	4.0	0.0	1.8	4.0	1.2	0.4	0.6	12.0
31	1.9	0.4	1.5	2.1	6.2	0.8	0.2	13.1
31	0.6	0.2	1.2	3.7	6.3	1.0	0.1	13.1
31	1.2	0.9	1.8	4.3	2.2	3.8	0.5	14.7
29	1.7	0.2	2.3	3.5	2.5	2.1	1.0	13.3
29	3.4	0.3	5.1	5.9	2.5	0.5	1.5	19.2
29	2.0	0.0	1.9	4.4	2.0	1.7	1.4	13.4
29	1.5	0.2	4.5	3.5	1.5	1.0	0.8	13.0
29	1.7	0.0	3.5	2.5	1.5	0.6	1.7	11.5
31	2.0	1.2	2.4	4.3	3.4	1.5	0.9	15.7
31	2.3	1.7	1.9	4.6	3.7	2.1	1.1	17.4
32	1.5	3.0	1.9	5.7	4.7	3.9	1.8	22.5
32	1.2	2.6	1.7	4.7	5.0	2.7	1.1	19.0
32	1.7	2.9	1.8	4.7	5.1	2.0	2.3	20.5
32	1.2	1.2	1.3	4.8	6.1	2.4	1.7	18.7
32	1.2	0.0	1.3	3.6	3.6	0.9	0.2	10.8
32	1.3	0.2	1.3	3.7	2.8	1.3	0.3	10.9
32	2.0	2.5	1.7	3.9	4.4	2.1	0.6	17.2
32	0.6	0.9	1.1	3.7	4.2	1.4	1.1	13.0
32	1.6	2.7	2.3	3.9	4.0	1.3	0.1	15.9
32	1.4	3.2	1.3	4.7	3.0	1.3	0.6	15.5
	1.0	0.0	1.0	1.8	3.0	2.3	0.7	9.8
	—	0.0	0.7	2.1	2.3	1.7	1.1	7.9
30	0.5	0.0	0.6	0.8	1.0	1.3	1.1	5.3
30	0.4	0.2	0.2	0.5	1.0	1.1	1.0	4.4
30	0.7	0.2	0.5	1.5	0.4	1.1	1.4	5.8
30	1.7	0.2	0.5	2.0	0.6	1.0	1.4	7.4
30	2.0	0.0	0.6	1.7	1.9	0.8	1.3	8.3
30	2.1	0.0	0.3	1.8	2.4	1.0	1.1	8.7
30	1.7	0.7	1.0	1.7	2.8	2.0	1.4	11.3
30	1.4	0.8	1.1	1.9	2.6	1.6	1.7	11.1
30	1.0	0.4	0.2	1.5	2.1	2.2	1.3	8.7
30	2.1	0.0	0.2	2.9	2.0	0.8	1.2	9.2
30	1.6	0.0	0.5	2.2	0.9	0.7	1.0	6.9
30	0.8	0.4	0.7	1.2	1.0	0.8	0.5	5.4
30	0.7	0.2	0.2	1.1	0.7	0.6	0.5	4.0
30	0.9	0.1	0.3	1.3	1.1	0.7	0.5	4.9
30	1.1	0.3	0.5	0.8	0.8	0.7	0.6	4.8
30	1.4	0.7	0.7	2.5	1.6	1.5	0.8	9.2
30	0.8	0.5	0.7	1.2	1.9	2.0	0.8	7.9
30	1.2	0.3	0.4	1.3	1.3	1.7	0.5	6.7
30	2.3	0.5	0.5	1.4	2.2	1.8	0.9	9.6
30	1.1	0.8	0.5	2.0	1.6	1.2	0.8	8.0
30	1.8	0.7	0.6	2.4	1.7	1.2	0.9	9.3
30	2.6	0.0	1.3	1.1	2.8	1.3	0.5	9.6
30	1.8	0.0	0.6	1.3	2.7	0.9	0.7	8.0
30	2.0	0.0	0.6	2.2	3.6	1.3	0.8	10.5
30	2.1	0.0	0.9	1.7	2.7	2.4	0.6	10.4
30	1.4	0.0	0.7	2.3	1.2	1.9	0.8	8.3
30	1.5	0.0	0.8	1.5	2.6	1.3	1.0	8.7
30	1.1	0.0	0.7	2.7	2.1	1.5	1.1	9.2
30	1.9	0.0	1.1	1.4	3.0	1.6	0.8	9.8
30	2.5	0.5	0.5	1.4	3.1	1.6	1.0	10.6
30	1.1	0.0	0.9	1.2	1.5	1.4	0.6	6.7
30	1.7	0.6	0.3	0.9	2.5	1.6	0.8	8.4
30	2.4	0.9	1.1	1.4	2.5	1.5	1.0	10.8
30	2.5	0.8	0.8	1.6	2.1	1.5	0.9	10.2
30	2.1	0.8	0.9	1.1	1.8	1.0	0.7	8.4
32	0.0	0.0	0.1	0.5	0.9	0.7	0.1	2.3
33	0.1	0.2	0.6	1.7	0.4	0.3	0.2	3.5
32	1.9	2.1	1.9	5.6	5.2	2.1	0.6	19.4
32	1.9	2.1	2.0	5.7	5.3	1.9	0.6	19.5
32	2.2	2.3	1.9	5.6	5.4	1.3	1.5	20.2
33	1.8	2.7	1.5	2.0	3.8	1.8	1.1	14.7
36	0.9	0.2	0.2	1.2	1.2	1.0	0.3	5.0
36	1.6	0.4	1.2	3.1	2.8	1.5	0.9	11.5
41	0.6	0.0	1.0	2.0	0.4	0.6	0.5	5.1
41	0.5	0.0	0.8	1.2	1.5	0.6	1.1	5.7
41	0.5	0.0	0.6	1.3	1.1	0.9	0.7	5.1
46	0.7	1.3	1.3	3.1	3.1	2.0	0.3	11.8
X_i =	111.4	48.00+90.5		201.2	199.9	110.2	64.2	825.4
X_i^2 =	206.20	405.97		694.20	696.39	193.66	69.42	10,584.40
$\bar{X}$ =	1.47	1.82		2.65	2.63	1.45	0.84	10.86
σ =	0.75	1.43		1.47	1.51	0.67	0.45	4.65

TABLE 3. NORMAL MEN

Age	17-Ketosteroids in mg./24 hrs.							
	I Non-alcoholic 17-keto-steroids	II *i*-Andro-stanol-6-one-17	III *β*-Keto-steroids	IV Andro-sterone (+Δ9-andro-stenolone)	V Etiocholan-olone (+Δ9-etiocholen-olone)	VI+VII 11-Hy-droxy-17-keto-steroids	VIII Uniden-tified fraction	Total
18	1.2	0.0	0.7	5.8	1.1	2.3	1.4	12.5
18	1.8	0.0	8.6	9.5	4.7	2.7	1.4	28.7
19	1.0	3.0	2.1	3.8	4.9	1.6	0.3	16.7
22	1.4	1.2	0.8	3.8	2.9	1.9	0.5	12.5
24	2.9	0.0	3.0	6.8	6.6	2.0	1.2	22.5
24	4.3	0.0	3.5	4.6	8.2	3.0	1.3	24.9
29	1.8	0.5	2.7	3.2	2.0	1.2	2.9	14.3
30	2.0	5.1	2.1	6.1	5.0	2.0	1.8	24.1
30	1.5	7.0	4.4	8.6	6.0	2.8	1.0	31.3
33	1.5	0.1	1.6	3.0	1.6	1.2	0.9	9.9
36	0.9	2.3	3.8	6.6	4.3	1.5	0.2	19.6
37	1.5	0.0	4.9	5.8	2.6	3.3	1.3	19.4
37	1.8	3.7	2.4	8.5	3.4	2.5	0.7	23.0
37	1.7	0.7	3.8	3.9	2.4	3.4	0.8	16.7
37	1.9	0.0	2.8	6.3	2.4	3.8	1.8	19.0
37	2.4	0.8	3.6	8.6	3.6	2.2	0.6	21.8
37	2.5	5.2	3.9	6.1	3.7	3.0	1.9	26.3
37	1.7	1.8	3.5	6.1	4.0	1.6	2.0	20.7
37	1.4	1.1	2.3	3.9	2.5	2.8	2.4	16.4
37	1.3	2.3	3.0	5.9	3.3	4.4	0.5	20.7
37	1.0	3.5	3.0	5.6	3.5	1.5	1.2	19.3
37	1.9	4.3	3.3	6.6	3.7	2.3	2.4	24.5
37	1.3	2.5	3.7	5.5	3.2	3.8	1.5	21.5
37	1.9	3.9	3.5	7.8	4.9	3.1	0.3	25.4
37	2.1	3.6	3.1	2.5	3.8	3.5	1.0	19.6
37	1.6	4.9	4.5	8.2	3.5	2.7	0.2	25.6
39	0.4	1.9	1.3	2.7	3.0	2.2	1.4	12.9
41	1.3	0.0	1.5	4.3	4.0	1.8	0.1	13.0
	2.5	0.0	2.5	4.8	3.9	2.3	0.2	16.2
X_i =	50.5	59.4	89.9	164.9	108.7	72.4	33.2	579.0
X_i^2 =	102.61	962.27		1,041.45	469.29	199.96	53.52	12,344.14
$\bar{X}$ =	1.74	5.15		5.69	3.75	2.50	1.14	19.97
σ =	0.72	2.63		1.93	1.49	0.8	0.7	5.29

In all our data on the analysis of urine extracts, those cases in which the Pincus reaction was applied are specially indicated.

RESULTS

17-Ketosteroid determinations on the urine of normal male and female adults.

The analysis described in the foregoing sections was applied to urine extracts from normal test subjects: 13 women aged 18 to 46 years and 14 men aged 18 to 41 years. On some individuals repeated analyses were made in order to provide information on the daily variations in excretion in a single individual. These variations were found to be so large that, when the results are statistically treated, the repeated determinations on a single subject have the same significance as the same number of determi-

nation on different subjects. Analyses were carried out on 76 female[4] and 29 male urines. The results are shown in Tables 2 and 3.

The mean total 17-ketosteroid content of the urine of the women was 10.9 mg. per twenty-four hours (standard deviation 4.65). From this we see that a value of more than 20 mg. per twenty-four hours in a woman must be regarded as unduly high. An unduly low excretion in a woman would be represented by a value below 1.5 mg. per twenty-four hours.

For the men the mean value found was 20.0 mg. per twenty-four hours (standard deviation 5.29). This means that the value for a normal male subject may vary between 9.4 and 30.6 mg. per twenty-four hours.

A fact worthy of note is the very wide variation in the excretion of *i*-androstanolone (II) in both sexes. This substance is transformed by heating with mineral acids into dehydroepiandrosterone (4, 5). For statistical purposes it was, thus, more correct to add the amounts of (II) (*i*-androstanolone) and III (β-17-ketosteroids). It was, in fact, found that the standard deviation of the sum of II and III was considerably smaller than that of the separate values.

For most of the compounds investigated, the standard deviation found for male test subjects was smaller than that for females. This is undoubtedly due to the fact that fewer analyses were performed on the male subjects.

The Pincus reaction was applied to some of these extracts of normal urine for further differentiation of the maxima III, IV and V. These results are shown in Table 4.

TABLE 4

Sex	Pincus: in mg. androsterone / Zimmermann: in mg. 17-ketosteroid		
	III	IV	V
F	0.60	0.96	0.92
F	0.67	1.00	1.00
F	0.73	1.06	—
F	—	0.80	0.91
F	—	1.00	—
F	0.69	0.87	0.94
M	0.43	0.95	0.87
M	0.71	0.88	0.95
M	0.29	0.81	—

[4] Of these 76 analyses, 42 were performed according to our method by Mrs. J. de Mol van Otterloo-Hülsman in the laboratory of the Antoni van Leeuwenhoekhuis in Amsterdam.

Here we see that in our normal subjects there is a marked quantitative predominance of androsterone and etiocholan-3(α)-ol-17-one in the maxima IV and V, whereas in fraction III the unsaturated dehydroepiandrosterone predominates.

The data shown in Table 4, although few, have convinced us that the values as shown in Tables 2 and 3 do not undergo any fundamental alterations when the Pincus method is used for further differentiation of the maxima IV and V. We believe that the values shown in Tables 2 and 3 may continue to serve as a basis for our further investigations, maximum IV then being ascribed to androsterone and maximum V to etiocholanolone.

We propose the following definition of the normal 17-ketosteroid excretion pattern for adults:

Women:

			Per cent
Total 17-ketosteroid content: 1.5–20.0 mg./24 hrs.	I	(nonalcoholic 17-ketosteroids)	1–27
	II+III	(*i*-androstenolone+β-17-ketosteroids)	1–29
	IV+V	(androsterone+etiocholanolone)	30–66
	VI+VII	(11-hydroxy-17-ketosteroids)	3–26
	VIII	(unidentified 17-ketosteroids)	0–19

Men:

		Per cent
Total 17-ketosteroid content: 9.6–30.6 mg./24 hrs.	I	2–16
	II+III	8–41
	IV+V	33–63
	VI+VII	4–23
	VIII	0–15

DISCUSSION

The 17-ketosteroid mixture in the urine of men is found to have about the same percentage composition as that in the urine of women, except for the percentages of *i*-androstan-6-ol-17-one and of β-17-ketosteroids, which seem to be higher in the male.

Opinions still differ as to the source of the urinary 17-ketosteroids. The majority of the authors support the view, that the 17-ketosteroids in the urine of women are exclusively metabolites of adrenocortical hormones. The 17-ketosteroids in the urine of men are regarded as metabolites of adrenocortical hormones and of the testosterone from the male gonads. A strong argument for this hypothesis is found in the fact that the excretion of 17-ketosteroids is very low in the urine of female patients with Addisons' disease.

This hypothesis fails, however, to provide a satisfactory explanation of the apparent similarity of the 17-ketosteroid pattern of the urine of males and of females. If the higher amount in the urine of males were due

to testosterone secreted by the testes, one should expect the surplus would be present in the form of metabolites of testosterone (androsterone and etiocholanolone) but this is not the case. On the contrary, the substances present in a higher percentage in the urine of males, *i*-androstanol-6-one-17 and β-17-ketosteroids, are certainly of adrenal origin.

In the case of the male the following facts are known with certainty:

a) Part of the 17-ketosteroid content of male urine is formed by metabolites of the gonadal hormone.

b) Metabolites of the gonadal hormone of the man are in particular androsterone and etiocholanolone. This was ascertained by Callow and co-workers (10) and by several other authors.

c) The urine of male castrates continues to contain small amounts of androsterone and etiocholanolone. Presumably the androsterone and etiocholanolone in the urine of males are not solely of gonadal origin. If the hypothesis is retained that the 17-ketosteroids of the female have their origin exclusively in the adrenal cortex, it has to be assumed that the adrenal cortex of the female behaves—as regards the formation of precursors of 17-ketosteroids—differently from the adrenal cortex of the male (11).

An alternative explanation may be that the female gonads secrete substances which undergo transformation to the neutral 17-ketosteroids, androsterone and etiocholanolone. The fact that the total 17-ketosteroid excretion of ovariectomized women remains with the limit of normality is not necessarily incompatible with this view. It should be remembered that the normal excretion in women varies from 1.5 to 20.0 mg. per twenty-four hours. Of this, 30 to 66 per cent is accounted for by androsterone and etiocholanolone. Even in total absence of these metabolites, a total excretion of 0.5–14 mg. per twenty-four hours would remain. In addition to this, we know that castration in the female does not lead to a total absence of these substances in the urine; Hirschmann (12) isolated androsterone and etiocholanolone from the urine of female castrates. This means that we may expect to find total 17-ketosteroid values even higher than 14 mg. per twenty-four hours in the urine of ovariectomized women. The hypothesis of the ovarian origin of part of the androsterone and etiocholanolone in the urine of normal women does not, therefore, conflict with the data reported in the literature, dealing with the total 17-ketosteroid excretion of female castrates. Hirschmann stated that the androsterone and etiocholanolone content in the urine of female castrates is only *slightly lower* than the values found by Callow in the urine of normal women. Thus, a large series of analyses of urine of young female castrates will be required to obtain any definite information on the existence of an ovarian precursor of neutral urinary 17-ketosteroids.

The publication of data on 17-ketosteroid excretion in children, adults with hypogonadism, and castrates will shortly provide us with the opportunity of discussing the problem in more detail.

The consideration of pathologic conditions, such as Cushing's syndrome and the adrenogenital syndrome, will pave the way to further discussion of the origin of the other "urinary 17-ketosteroids" previously mentioned.

SUMMARY

1. A method for the quantitative analysis of 17-ketosteroids in the 24-hour urine specimen is described.

2. This method has been applied to urine samples obtained from 13 women between the ages of 18 and 46 years and 14 men between 18 and 41 years. Determinations were made on 76 samples of female urine and 29 samples of male urine.

3. The values found for the total 24-hour excretion in women ranged from 1.5 to 20.0 mg., and in men, from 9.4 to 30.6 mg. The mean values were 10.9 mg. for women and 20.0 mg. for men.

4. The percentage composition of the mixture of 17-ketosteroids is the same in both sexes, except for the percentages of *i*-androstanolone and β-17-ketosteroids.

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A CRITICAL ANALYSIS OF THE QUANTITATIVE I^{131} THERAPY OF THYROTOXICOSIS*

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INTRODUCTION

DESPITE the wide clinical use of radioactive iodine, I^{131}, in the treatment of thyrotoxicosis, agreement has not been attained concerning dosage or schedule of administration. Inadequacy of methods for the measurement of radiation delivered by I^{131} to the thyroid gland has presented a major obstacle to quantitative dosage. There has been, in consequence a paucity of data as to the amount of I^{131} radiation required for effective treatment.

To achieve quantitative dosage with I^{131} it is necessary to deliver a predetermined amount of radiation to the thyroid gland. The radiation delivered to the thyroid by I^{131} is a function of the administered dose, its uptake and turnover by the gland and the weight of the gland (1). Assuming a constant weight of the thyroid, the possibility of delivering a predetermined radiation dosage is predicated upon the reliability of a tracer dose of I^{131} as an indicator of the metabolism of a therapeutic dose. Utilizing the method (2, 3) developed by us for measurement of the I^{131} content of the thyroid, we are investigating the possibility of treating thyrotoxicosis by the delivery of calculated amounts of radiation with I^{131}. The present report is concerned with the following problems: 1) Is the uptake and turnover (biologic half-life) following a therapeutic dose of I^{131} predictable from the uptake and turnover following a tracer dose? 2) What is the relationship between the estimated radiation delivered by the therapeutic dose of I^{131} and the therapeutic effect?

REVIEW OF LITERATURE

The earliest series of thyrotoxic patients treated with radioiodine was reported without detail in 1942 (4, 5). In 1946 Hertz and Roberts (6) reported the "cure" of 20 of 29 patients treated with cyclotron-produced radioactive iodine (I^{130} and I^{131}); stable iodide was administered from one

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TABLE 1. SUMMARY OF RESULTS OF TREATMENT OF THYROTOXICOSIS WITH I^{131} IN OVER 1,000 CASES

TABLE 1. SUMMARY OF RESULTS OF TREATMENT OF THYROTOXICOSIS WITH I^{131} IN OVER 1,000 CASES

Author	Number of cases	Factors used in estimation of radiation				Administered dose			Results				No. of doses (No. of patients receiving)		
		Direct measurement of I^{131} uptake by thyroid gland	I^{131} excretion in urine	Biologic half-life	Estimation of size of thyroid gland	Millicuries	Microcuries per gram	Thyroid radiation, REP	Euthyroidism	Persistent thyrotoxicosis	Hypothyroidism	Inomplete	One	Two	More
Chapman, Evans (8)	135	No direct measurement; estimated from urinary excretion	48-hour collection	Not reported	Normal assumed to weigh 20 Gm.	6–15	Range[1] 100–200	Not stated	107[2]	8	8	10	104	11	
Werner, Quimby, Schmidt (9)	103	Single G-M tube at 15 cm.; within 24 hours	To supplement uptake studies	4–7 days (avg. 6). Determined experimentally by weekly readings	Palpation compared to plasticene models	3–6.5 per dose	50–250[3]	1,000 to >10,000[4]	85	8	4[5]	6	73	25	5
Haines, Keating, Power, Williams, Kelsey (10)	40	No direct measurement; estimated from urinary excretion	72-hour collection	Not measured	Not stated	2.6–20	Not stated[6]	Not stated	25[7]	6	7	2	29	10	1
Crile, McCullagh, Glasser (11)	105[8]	One reading by single G-M tube 2 to 4 hours after dose	Not measured	Not measured	Not stated	Initial dose: 4–8, diffuse goiter 8–15 nodular goiter	Not stated	Not stated	17 5	2 5	2 0		12 5	8 3	1 2
Prinsmetal, Agress, Bergman, Simkin (12)	Over 100;[9] 26 Graves' disease; 18 problem cases	Single G-M tube at 29 cm.; within 48 hours	Not measured	Not measured	Not stated	0.5–10 1.5–14.5	Not stated	Not stated	25 16	0 2	1				
Soley, Miller, Foreman (13)	68 cases; 46 in detail	Single G-M tube at 53 cm.; on first 3 days	Collected	Not measured	Compared with weight observed at operation	1–4 reported	21.3–247.8; avg., 77.7	Not stated	40	4	2[10]				
Williams, Towery, Jaffe, Rogers Tagnon (14)	106[11]	Single G-M tube at 1 cm., successively over each lateral lobe	Collected for more than 24 hours	Not measured	Palpation[12]	2–36; 2–8 in 32 cases responding well to one dose	225	Not stated	89	4	3	1	33	28	36
Feitelberg, Kaunitz, Silver, Simon, Wasserman, Yohalem (15)	290; 184 in detail	No direct measurement; estimated from urinary excretion	Collected	Assumed 6 days	Estimated by 3 observers	Average, 8.2; maximum initial dose 11.0	80 taken up	10,000	169	2	13		132	43	9
Gordon, Albright (16)	120	Single G-M tube; within 24 hours	Collected for 72 hours initially; later omitted	Not reported	Not estimated; dose not related to gland weight	1.5–20.5[13]	Not stated	Not stated	112	0	3	5	59	36	25
Moe, Adams, Rule, Moore, Kearns, Clark (17)	100[14]	Single G-M tube; within 24 hours	Not collected	Not measured	Not stated	Diffuse, 1.1–17.8 nodular, 2.2–43.9	100	Not stated	88	0	12		46	28	26
Freedberg, Kurland, Chamovitz, Ureles	42	4 G-M tubes; at 24 hours and several times thereafter	Collected for 24 to 72 hours or longer	Measured 3–14 days	Average of 5 observers, normal 25 Gm.	2.3–25.0	Not stated	5,800–37,500	25	1	8	8	26	5	3

[1] 160 μc./Gm., suggested dose.
[2] Average, 110 μc./Gm. retained by patients rendered euthyroid. 130μc./Gm. retained by patients rendered hypothyroid.
[3] Eight patients received more than 200 μc./Gm.
[4] Attempted to deliver 5,000 REP. Highest remission rate at 7–10,000 REP.
[5] Thyroid radiation in these cases, 11,800; 8,200; 7,700; 2,690 REP.
[6] Attempted to administer a dose to allow thyroid collection of 200–250 μc./Gm.
[7] Thyroid retention in patients with good results, 87–574, avg. 242 μc./Gm. Myxedema produced by 158–522, avg. 283 μc./Gm. Three recurrences.
[8] Thirty-four patients followed 3 mos.
[9] Forty-four patients followed 1 yr. Tracer dose 0.5 to 1.0 millicurie. One relapse. Additional dosage proportionate to BMR.
[10] Hypothyroidism from uptake of 43.5 and 47.5 μc./Gm.
[11] Ninety-seven patients followed 3 mos.
[12] Average error in previous series stated to be 18 per cent.
[13] Multiple treatments by doses consistently too small.
[14] Tracer dose, 1 millicurie. Additional therapy 8 wks. after initial dose, proportionate to residual toxicity.

day to several weeks after the radioactive iodine. No direct measurement of the thyroid uptake of radioactive iodine was made; the thyroid uptake was estimated from the urinary excretion. The range of delivered thyroid radiation varied from 210 to 4,300 roentgen equivalents physical,[1] but an error of 50 per cent or more was assumed and ascribed to errors in estimation of gland weight and uptake and turnover. Chapman and Evans (7, 8) reported the results of the treatment of 65 thyrotoxic patients with I^{130} and I^{131}; stable iodide was not administered. Estimations of thyroid radiation, utilizing urine excretion measurements as an indication of thyroid uptake, showed that a dose of from 5 to 15,000 REP was delivered. Fifty of the 65 patients were restored to a euthyroid state; 11 became hypometabolic; and in 3, persistent hyperthyroidism was observed.

Since 1946, I^{131}, derived from the atomic pile and obtained from the Atomic Energy Commission, has been utilized in the treatment of more than 1,000 cases of thyrotoxicosis (Table 1) (8–17). The wide variety of methods utilized to measure the thyroid gland I^{131} uptake and turnover is notable (Table 1). Some investigators have estimated the thyroid gland uptake from the urinary excretion; others have employed direct measurement by external counting methods; the techniques, however, have varied from clinic to clinic. In most instances, the biologic or effective half-life[2] (1, 18) is omitted in dosage determination. Haines *et al.* (10) have stated that the biologic half-life following a therapeutic dose cannot be predicted with certainty from the measurement of the biologic half-life following a tracer dose. Werner *et al.* (9) have, however, utilized this factor in the calculations of thyroid radiation and have determined it for each case after therapeutic dosage. In other investigations, the biologic half-life has been assumed to be six days (15). The difficulty of estimation of the size of the thyroid gland by physical examination has been stressed (9, 10, 13, 16). The efforts of various investigators to reduce the size of this error have included estimation of thyroid gland size by comparison with plasticene or rubber models of known size, or with the weight of thyroid glands obtained after surgical subtotal thyroidectomy.

As a result of the wide variety of techniques, wide divergence exists in the mode of measurement and expression of I^{131} dosage (8–17). In some reports the estimated roentgen equivalents delivered to the thyroid gland are recorded; in others, there is only a statement of the millicuries adminis-

[1] One REP is defined as an amount of radiation (other than protons) which releases in tissue an amount of energy equal to the energy loss of one roentgen of gamma radiation absorbed in air. More recently it has been demonstrated that one roentgen has been delivered to a tissue when it has absorbed 93 ergs.

[2] Biologic or effective half-life of the isotope in tissue includes the rate of elimination from the tissue and the physical rate of disintegration of I^{131}.

tered or the millicuries administered per gram of thyroid gland, with or without adjustment for thyroid gland uptake, Some groups (9, 10, 15) have sought to produce a euthyroid state with a single dose, whereas others have preferred repeated small doses (11, 16). Despite the wide variety of methods used, the reported results have been good, so that radioactive iodine has become a valuable agent in the treatment of thyrotoxicosis.

MATERIALS AND METHODS

Forty-two patients, 36 female and 6 male, aged 16 to 72 (average 43) were studied. In all instances, the diagnosis of thyrotoxicosis was made on the basis of the characteristic symptoms, signs, and laboratory findings. The latter included I^{131} tracer studies, measurement of the serum cholesterol and serum protein-bound iodine, and repeated measurements of the basal metabolic rate.

Technique of I^{131} studies

Radioactive iodine, I^{131}, was allocated by the Atomic Energy Commission and obtained from Oak Ridge, Tennessee. The millicurie strength was calibrated by Tracerlab, Inc., Boston, and independently by us and corresponds to the strength of the millicurie of the National Bureau of Standards. Tracer doses of 100–150 microcuries of carrier-free I^{131}, measured volumetrically, were administered to patients in the fasting state at least fourteen hours after the previous meal. The dose was first diluted with approximately 50 cc. of tap water. The glass was rinsed two or three times with 50 cc. of tap water, and the rinsings were also administered. (The residual I^{131} in the glass has been measured and found to be negligible.) The uptake of the administered dose of I^{131} by the thyroid gland was determined directly by the four-tube method previously described (2, 3). The formula employed was:

$$\text{Thyroid gland uptake } \% = \frac{\text{net counts/min. in thyroid gland}}{\dfrac{\text{cc. } I^{131} \text{ solution administered}}{\text{cc. } I^{131} \text{ solution in standard}} \times \text{net counts/min. standard}} \times 100.$$

Measurements were made twenty-four hours after the administration of the tracer dose and on at least two occasions thereafter, during the next seven to ten days. In most cases more than three measurements were obtained. The biologic or effective half-life was calculated by plotting the net thyroid counts as the ordinate against days as the abscissa on semi-log paper; a straight line was drawn which best fitted the plotted points (Fig.

TABLE 2. COMPARISON OF THYROID UPTAKE, TURNOVER AND RADIATION FOLLOWING TRACER AND THERAPEUTIC DOSES OF I^{131}

Patient				Radioactive iodine, I^{131}														
Case No.	Initials, age, sex	Group	Wt. of thyroid gland (Gm.)	Thyroid gland uptake, %			Biologic half-life (days)			24-Hour urine excretion, %		Dose, in millicuries	Thyroid radiation (REP)			Duration of follow-up, months	Results	Comment
				Tracer	Ther.	% Diff.	Tracer	Ther.	% Diff.	Tracer	Ther.		Anticipated	Actual	Diff.			
1	M.L. 54 F	A A	70 65	64 60	77 50	+20 −16	7.0 5.6	8.0 5.5	+14 − 1.8	17	34	7.1 8.0	9,100 8,300	12,500 6,800	+37 −19	2 12	Improved Hypothyroid	KI until two wks. before tracer
2	G.S. 40 F	A	20	50	52	+ 4	6.5	6.2	− 7.7			2.8	9,100	9,000	− 1	18	Euthyroid	
3	I.L. 43 F	B B	50 30	83 54	87 59	+ 4.5 + 9.3	8.0 5.0	6.4 4.3	−20 −14	34	32	3.3 2.3	8,700 4,100	7,400 3,900	−15 − 5	6 12	Improved Euthyroid	
4	B.M. 45 M	B	45	54	61	+13	5.5	4.0	−27			8.7	11,500	9,400	−18	18	Hypothyroid	KI until 3 mos. before tracer
5	A.R. 59 F	A B	60 40	83 64	83 64	0 0	4.8 5.6	5 4.6	− 4.2 −18			7.9 4.4	10,300 7,800	10,500 6,500	+ 2 −17	4	Improved Hypothyroid	KI until 3 wks. before tracer
6	G.B. 37 M	B B	50 30	74 52	73 45	− 1.2 −13.5	5.5 3.7	5.2 4.2	− 5.5 +14	22.5 38	18.5 43	5.3 4.2	8,600 5,500	8,000 5,600	− 6.5 + 1.8	7 12	Improved Euthyroid	
7	H.L. 57 M	B	35	78	80	+ 2.3	6.1	6.0	− 1.7	10	8.5	3.4	9,200	9,200	0	19	Euthyroid	
8	C.L. 16 F	B B	80 55	88 74	89 72	+ 1.1 − 2.7	6 3.4	3.4 2.7	−43 −20	9.5	9	6.5 12	8,600 11,000	5,000 8,500	−42 −23	4 14	Unchanged Euthyroid	
9	D.P. 36 F	B	55	71	72	+ 1.4	6.2	6.4	+ 3.2	29	27	5	8,000	8,300	+ 3.5	19	Hypothyroid	
10	E.C. 61 F	B	45	77	77	0	6.2	6.2	0	21	18	4.9	10,400	10,400	0	19	Euthyroid	
11	H.S. 58 F	B	55	78	86	+12	5	5	0	15.5	10.5	6.4	8,800	9,900	+12.5	19	Hypothyroid	
12	J.K. 42 F	A B B	80 75 45	82 80 86	81 83 76	− 1.1 + 3.5 −11.5	4.3 5.0 4.6	4.8 5.0 6.0	+12 0 +30	8.7 14	7.5 6	12. 10 5.3	10,000 10,700 9,200	11,000 11,000 10,800	+10 + 2.8 +17	3 2½ 16	Improved Mildly thyrotoxic (persistent)	KI until 3 wks., and propylthiouracil until 8 days before tracer

TABLE 2.—*Continued*

Patient				Radioactive iodine, I^{131}													
Case No.	Initials, age, sex	Group	Wt. of thyroid gland (Gm.)	Thyroid gland uptake, %			Biologic half-life (days)			24-Hour urine excretion, %		Dose, in millicuries	Thyroid radiation (REP)			Duration of follow-up, months	Results	Comment
				Tracer	Ther.	% Diff.	Tracer	Ther.	% Diff.	Tracer	Ther.		Anticipated	Actual	Diff.			
13	L.M.	A	100	84	86	+ 2.4	6	6	0	13	11	6.7	6,700	6,900	+ 3	3½	Improved	KI until 15 days and propylthiouracil until 10 days before tracer
	33	B	85	61	63	+ 3.3	3.6	2.8	−22	26	24	19.7	10,200	8,200	−20	4	Improved	
	F	B	45	45	50	+ 9	2.8	3	+ 7			13.5	7,600	9,000	+18	14	Euthyroid	
14	R.S.	A	120	87	86	− 1.1	5.0	5.8	+16	5	7	9.3	6,800	7,800	+15	3½	Improved	Propylthiouracil until 8 days before tracer. A fourth dose of 14,000 REP has resulted in euthyroid state
	33	B	115	89	86	− 3.3	5.4	5.2	− 3.7			8.1	6,800	6,300	− 7.4	3½	Improved	
	F	B	100	90	85	− 5.5	5.8	4.8	−17	6.4	5.8	11.5	11,700	9,400	−19.5	8	Improved	
15	I.M.	B	50	52	53	+ 1.9	4.8	5.4	+12.5			8.5	8,400	9,700	+15.5	18	Euthyroid	
	72																	
	F																	
16	C.F.	B	?*	58	58	0		7.8				2.7				16	Euthyroid	Four previous thyroidectomies. Thyroid gland not palpable
	63																	
	F																	
17	T.S.	B	75	75	75	0	3.6	3.5	− 2.8	13	11	9.0	6,500	6,300	− 3	15	Euthyroid	Persistent thyrotoxicosis after 2 previous doses of I^{131}, 5 mc. each
	23																	
	F																	
18	F.L.	A	100	71	76	+7	6.0	6.5	+ 8.5	14	10	17.3	14,700	17,000	+15	16	Euthyroid	KI until 3 wks. before tracer
	51																	
	F																	
19	R.M.	A	75	69	86	+24.6	4.0	4.6	+15	12	8	18.0	13,300	19,100	+43		Hypothyroid	Tracer 3 days after propylthiouracil
	24																	
	F																	
20	E.S.	A	60*	59	26	−56	5.0	5.0	0	12	55+	25	24,800	11,000	−53	24		I^{131}, 13.8 mc., 3 mos. previously. Cough syrup on day of I^{131} therapy
	60																	
	F																	
21	A.S.	A	60	64	63	− 1.6		4.5→ 2.5		10	11.4	13.5		10,300				Tracer 2 days after propylthiouracil
	12																	
	F																	
22	N.M.	A	50	81	32	−60	4.7	8→5.6				5.4	8,300	4,600	−45	16	Euthyroid	Intravenous pyelogram 2 days before treatment
	25	A	50		84			avg.6.6				2.3		3,900				
	F							5	40					8,500	+ 2.4			
23	S.K.	A	60	83	87	+ 4.8	4.4	4.4	0			7.1	8,600	9,100	+ 5.8	16	Euthyroid	Tracer 4 wks. after KI and propylthiouracil
	39																	
	F																	

TABLE 2.—*Continued*

Patient				Radioactive iodine, I^{131}														
Case No.	Initials, age, sex	Group	Wt. of thyroid gland (Gm.)	Thyroid gland uptake, %			Biologic half-life (days)			24-Hour urine excretion, %		Dose, in millicuries	Thyroid radiation (REP)			Duration of follow-up, months	Results	Comment
				Tracer	Ther.	% Diff.	Tracer	Ther.	% Diff.	Tracer	Ther.		Anticipated	Actual	Diff.			
24	R.B. 41 F	B	40	82	81	− 1.2	4.9	6.0	+22	16	15	4	8,000	9,700	+21	16	Euthyroid	
25	S.J. 73 F	B	40*	69	61	−12	6.0	5.0	−17	25	19	4	8,300	6,100	−26.5	16	Euthyroid	
26	F.K. 33 F	A A	50	74	69 18	− 6.8	5.0	3 1.7→ 5.4	−40	18	14	6.9 4.2	10,100	5,700 1,100 6,800	−43 −33	14	Euthyroid	"Double serving" of scallops 33 hrs. after treatment
27	C.O. 41 M	B	75	82	84	+ 2.4	6.1	6.6	+ 8.2	9	8.5	7.6	10,100	11,200	+11	14	Euthyroid	
28	S.C. 40 M	B	55	46	41	−11	6.4	6.0	− 6.3	48	50	9.8	10,500	8,600	−18	14	Hypothyroid	
29	A.M. 34 F	B	40*	73	68	− 6.9	5.1	6.1	+19.6	22	28	5.8	10,800	11,900	+10	13	Euthyroid	
30	N.K. 23 F	A	75	83	79	− 4.8	6.6	5.1	−23	10	7.5	6.9	10,100	7,500	−26	12	Euthyroid	
31	B.H. 32 F	B	55	84	76	− 9.5	5.4 (est.)	6.6		14.5	14	6.1	10,100	11,000	+ 9	13	Euthyroid	
32	B.K. 50 F	B	35	79	83	+ 5	5.6	5.2	− 7	11	12	4	10,100	9,900	− 2	12	Euthyroid	
33	P.G. 44 M	B	55	79	78	− 1.3	6.0	7.0	+16	10	4	6	10,400	12,000	+15.4	12	Euthyroid	
34	S.S. 55 F	B	45*	62	61	− 1.6	6.1	6.5	+ 6.5	41	30	6.2	10,300	10,800	+ 4.9	12	Euthyroid	

TABLE 2.—*Continued*

Patient				Radioactive iodine, I^{131}													
Case No.	Initials, age, sex	Group	Wt. of thyroid gland (Gm.)	Thyroid gland uptake, %			Biologic half-life (days)			24-Hour urine excretion, %		Dose, in millicuries	Thyroid radiation (REP)			Duration of follow-up, months	Results	Comments
				Tracer	Ther.	% Diff.	Tracer	Ther.	% Diff.	Tracer	Ther.		Anticipated	Actual	Diff.			
35	R.F. 35 F	A	30	76	72	− 5.3	3.0→ 5.2	1.8→ 3.4→ 3.8 est. 3.6		17	21	5.3	4,600	6,100	+24	12	Euthyroid	Previous dose of 5.6 mc. delivered 8,300 REP
36	J.H. 46 F	B	75	84	84	0	5.7	6.3	+10.5	13	3	8.0	10,100	11,300	+12	4½	Partial improvement	Euthyroid after additional dose of 2.3 mc.
37	L.R. 30 F	B	65	82	81	− 1.2	4.8	3.6	−25	3	1	8.4	10,100	7,500	−26	12	Euthyroid	
38	N.M. 58 F	A	40*	88	72	−18	7.4	7.0	− 5.4			3.2	10,400	8,000	−23	12	Euthyroid	
39	E.R. 57 M	B	45	83	84	+ 1.2	5.8	6.0	+ 3.4	10	11	4.7	10,100	10,500	+ 4	12	Euthyroid	
40	H.V. 35 F	A	65	92	94	+ 2	6.8	7.0†	+ 3	10	9	5.3	10,100	8,000	−20	12	Hypometabolic	
41	F.K. 61 F	A	50	48	30	−37	7.0	7.0	0	30	46	7.6	10,100	5,800	−43	10	Euthyroid	
42	A.G. 53 F	B	65*	60	49	−19	7.4	7.2	− 2.7	23	32	7.4	10,100	8,000	−20	2	Partial improvement	Paroxysmal tachycardia; congestive heart failure; hypometabolism subsequently induced intentionally by 16.5 mc. I^{131}

* Nodular goiter.
† Biologic half-life was 4 days, after the first week.

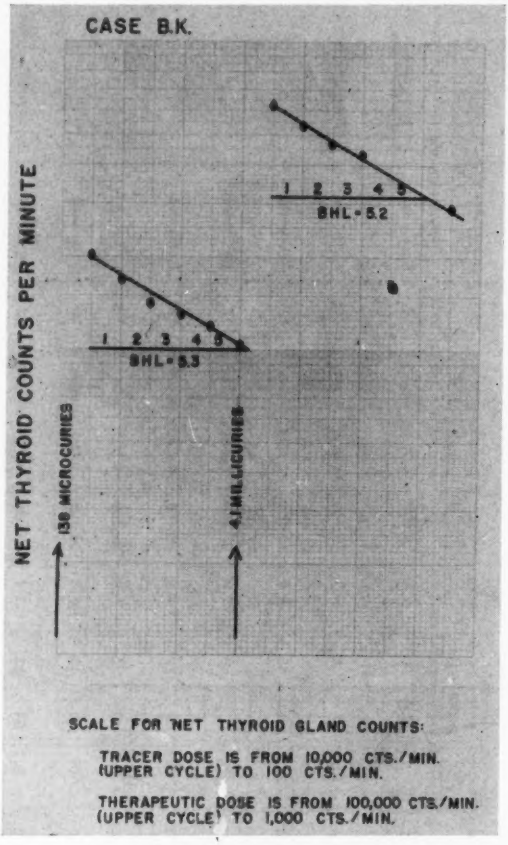
CASE B.K.
NET THYROID COUNTS PER MINUTE
1 2 3 4 5
BHL = 5.2
1 2 3 4 5
BHL = 5.3
130 MICROCURIES
4.1 MILLICURIES
SCALE FOR NET THYROID GLAND COUNTS:
TRACER DOSE IS FROM 10,000 CTS./MIN. (UPPER CYCLE) TO 100 CTS./MIN.
THERAPEUTIC DOSE IS FROM 100,000 CTS./MIN. (UPPER CYCLE) TO 1,000 CTS./MIN.

FIG. 1. Case 32, B.K. The biologic or effective half-life following tracer and therapeutic doses of I^{131}.

1). The biologic or effective half-life equalled the number of days required for the maximum net thyroid counts to decrease by 50 per cent. In almost all patients, the total urinary output was collected for one or more days in jugs containing from 6 to 8 pellets of sodium hydroxide and from 2 to 3 cc. of toluene. The I^{131} content of the urine was measured by the method of Marinelli and Hill (19) as modified by us (20) or by the four-tube method (21).

All patients were instructed concerning the common sources of iodine (22) to be avoided during the period of the study. This regimen was not rigidly adhered to by all patients. The effects of ingestion of iodide on I^{131} uptake and turnover are noted subsequently.

Calculation of thyroid radiation. In each patient the weight of the thyroid gland was estimated independently by 5 observers;[3] the average of the several observations was utilized in the calculation. The average difference of the extreme observations from the mean was ± 15 per cent. The normal thyroid gland was assumed to weigh 25 grams. The average weight of 30 normal thyroid glands obtained at autopsy at the Beth Israel Hospital was 23 grams.

The radiation to be delivered by the therapeutic dose was calculated from the formula (1)

$$\text{REP} = \text{dose (microcuries)} \times \text{uptake}\,\% \times \frac{\text{BHL}}{8}\text{(days)} \times \frac{160}{\text{weight of thyroid (grams)}}.$$

Following these studies, therapeutic doses of I^{131} calculated to deliver a predetermined number of REP were administered in similar fashion to the tracer doses and the observations were repeated. The percentage uptake of I^{131} by the thyroid gland, the biologic half-life, and the I^{131} excretion in the urine were measured and the actual radiation delivered to the thyroid gland was calculated. The patients were then followed in a special radiation clinic and observed at intervals of three weeks. At each visit clinical examination and various laboratory procedures, including measurement of the basal metabolic rate and basal pulse rate, were performed; when indicated, the I^{131} uptake and turnover, the serum protein-bound iodine and the serum cholesterol were determined.

RESULTS

In the analysis of the data we have examined the results obtained in the entire series of 53 comparisons of tracer and therapeutic doses in the 42 cases. This group of comparisons is subsequently termed "Group A." We have also analyzed and shall present separately 34 of these comparisons of tracer and therapeutic data obtained in 28 of the 42 cases. This latter group, subsequently termed "Group B," comprised all instances in which it was felt that ideal conditions existed for predicting the equivalent roentgens to be delivered by the therapeutic dose on the basis of the tracer data. The patients in Group B had received no treatment for thyrotoxicosis during the three months before I^{131} and the comparison of tracer and therapeutic data was not complicated by the intake of iodine-con-

[3] We wish to thank Dr. Mark F. Lesses and Dr. Samuel L. Gargill of the Endocrine Clinic of the Beth Israel Hospital for their help.

taining medication or food. The effects of the ingestion of iodide in food or medication are illustrated by Cases 22 and 26 (*see* section on illustrative cases).

The thyroid gland uptake

In Group A, the average uptake[4] of the tracer dose twenty-four hours after administration of I^{131} was 72.7 per cent (S.E., 1.8) and the average uptake of the therapeutic dose was 70.4 per cent (S.E., 2.4). The average difference[5] between the uptake measured after a therapeutic dose and that measured after the tracer dose in the same patient was ± 8.6 per cent (S.E., 1.7; S.D., 12.1). In 5 of the 53 comparisons (Cases 1, 19, 20, 22 and 41) the uptake of the therapeutic dose differed from that of the tracer dose by 20 per cent or more. In 4 of these 5 instances the probable source of the discrepancy was found (Table 2). In 1 patient (Case 1) in whom the therapeutic uptake was 20 per cent greater than the tracer uptake, stable iodide therapy had been discontinued only two weeks before the tracer dose. The therapeutic dose was administered three weeks after the omission of potassium iodide and the thyroid gland was still emerging from the effect of iodide in the interval between the tracer and the therapeutic doses (23, 24). One patient (Case 19) had omitted propylthiouracil only three days before the I^{131} tracer and seven days before the therapeutic dose. The uptake of the therapeutic dose was 25 per cent greater than following the tracer dose. Similar observations of increasing uptake from one to eight days after omission of propylthiouracil have been reported (3, 7). In the third patient (Case 20) cough syrup had been administered on the day of the therapeutic dose of I^{131}; the therapeutic uptake was 55 per cent lower than the tracer uptake. The fourth patient (Case 25, described hereafter in detail) had an intravenous pyelogram between the tracer and the therapeutic doses. In the remaining patient (Case 41) the therapeutic uptake was 37 per cent less than the tracer; no cause for this discrepancy was ascertained.

In Group B the average 24-hour uptake of the tracer dose was 71.9 per cent (S.E., 2.2) and of the therapeutic dose 71.3 per cent (S.E., 2.3). The average per cent difference of a therapeutic dose from its corresponding tracer dose was ± 5.0 per cent of the tracer dose (S.E., 0.9; S.D., 5.0). The ± 5.0 per cent difference observed in Group B is not significant statistically from the ± 8.6 per cent observed in Group A.

[4] Hereafter uptake refers to the measurement made twenty-four hours after the administration of I^{131}.

[5] The formula utilized was: —Per cent difference

$$= \frac{\%\text{ uptake tracer} - \%\text{ uptake therapeutic} \times 100}{\%\text{ uptake tracer}}.$$

Comparison of the urinary excretion and thyroid gland uptake

It was of interest to compare the thyroid gland uptake measured directly by the four-tube method with the theoretical thyroid gland uptake obtained by subtracting the fraction excreted in the urine during the first twenty-four hours from 100 per cent. In 38 such comparisons the average direct uptake following tracer doses was 73.3 per cent and the average indirect uptake was 82.5 per cent or, in absolute amounts, 9.2 per cent (S.E., 1.1; S.D., 6.9) more than the average direct measurement. The uptake estimated indirectly ranged from −3 to +26 per cent more than that obtained directly.

Similar findings were obtained following therapeutic doses in the same 38 patients. In 38 comparisons the average direct uptake following a therapeutic dose was 72.8 per cent and the uptake estimated indirectly was 81.9 per cent or 9.1 per cent (S.E., 1.3; S.D., 7.8) greater than the average direct measurement. The range of the differences of the uptake estimated indirectly was −5 to +26 per cent more than that measured directly. It should be stressed that only those comparisons have been included in which it was considered that the urine collection was complete. Six observations in 5 patients were discarded because urinary collection was inadequate. This is, of course, an ever present danger in a procedure requiring complete patient cooperation.

The biologic or effective half-life

In Group A (49 observations) the average biologic half-life of the tracer dose was 5.5 days (S.E. 0.17; S.D., 1.2) and the average biologic half-life following the therapeutic dose (50 observations) was 5.5 days (S.E., 0.15; S.D., 1.0). In this group the average difference (47 comparisons) between the biologic half-life measured after the therapeutic dose and that found after the tracer dose in the same patient was 11.5 per cent (S.E., 1.4; S.D., 9.8).

In 11 instances in 10 patients (Cases 3, 4, 8, 12, 13, 22, 24, 26, 30 and 37) (Table 2) the difference between the biologic half-life of a therapeutic dose and that of its corresponding tracer dose was 20 per cent or greater. In 8 of the 11 instances (Cases 3, 4, 8, 13, 26, 30 and 37) the biologic half-life of the therapeutic dose was shorter than that of the corresponding tracer dose; in the remaining 3 (Cases 12, 22 and 24) it was longer. In Case 22 (described subsequently) the difference is believed due to the effect of the administration of Diodrast between the tracer and therapeutic dose; no adequate explanation is available for the differences observed in the other cases.

In Group B (33 observations) the average biologic half-life of a tracer

was 5.4 (S.E., 0.12; S.D., 0.7) days and that of the therapeutic dose was 5.3 (S.E., 0.22; S.D., 1.3) days. In this group the average difference between the biologic half-life of the therapeutic dose and that of its corresponding tracer dose was 13.7 (S.E., 2.4; S.D., 13.7) per cent, not statistically different from Group A.

The roentgens equivalent physical (REP)

In Group A (50 comparisons in 40 patients) the REP delivered by the therapeutic dose differed from that anticipated on the basis of the tracer studies by an average of 16.8 per cent (S.E., 1.8; S.D., 13.2). The average difference in Group B (33 comparisons in 27 patients) was 12.9 per cent (S.E., 2.4; S.D., 13.6), not statistically different from Group A.

The differences between the anticipated and delivered radiation can be explained by deviations of the biologic half-life and the thyroid gland uptake following the tracer and therapeutic doses. In 17 instances in 16 patients the difference between the radiation anticipated on the basis of the tracer study and that delivered by the therapeutic dose was 20 per cent or greater (Table 2). In 9 of the 17 the discrepancy was largely due to a change in the biologic half-life; in 5 cases it was due to a change in the percentage uptake of the dose; in the remaining 3 cases, both factors varied.

In an additional 6 of the 50 comparisons the discrepancy between the anticipated and delivered roentgens would have been greater than 20 per cent if the thyroid uptake and biologic half-life had varied in the same direction. However, they varied in opposite directions and the resulting possible discrepancy in the radiation delivered was thereby reduced.

ILLUSTRATIVE CASES

Effect of intravenous Diodrast on the uptake and turnover of I^{131}

Case 22. BIH #7328, N. M., a 25-year-old woman, presented a characteristic history and the physical findings of thyrotoxicosis. For the previous three months she had noted increasing enlargement of the neck, prominent eyes, occasional diarrhea, oligomenorrhea, nervousness, heat intolerance, sweating, exertional dyspnea, palpitation, increased appetite and weight loss. The thyroid gland was diffusely enlarged and estimated to weigh 50 grams. The 24-hour thyroid gland uptake following a tracer dose of radioactive iodine was 81 per cent, the biologic half-life was 4.7 days (Fig. 2). Thirteen days after the tracer dose, Diodrast Compound was administered for visualization of the kidneys. Two days after intravenous pyelography, 5.4 millicuries of I^{131} was administered. On the basis of the tracer data it was anticipated that this dose would deliver 9,000 REP. However, only 35 per cent of the dose (1.9 millicuries) was taken up by the thyroid gland in the subsequent twenty-four hours. Furthermore, there was no turnover for the ensuing forty-eight hours (Fig. 2). During the subsequent forty-eight hours, the sixth and seventh days after Diodrast, the rate of decrease in measured thyroid radiation appeared similar to that of the tracer dose. Therefore, a second dose of 2.3 millicuries was administered five

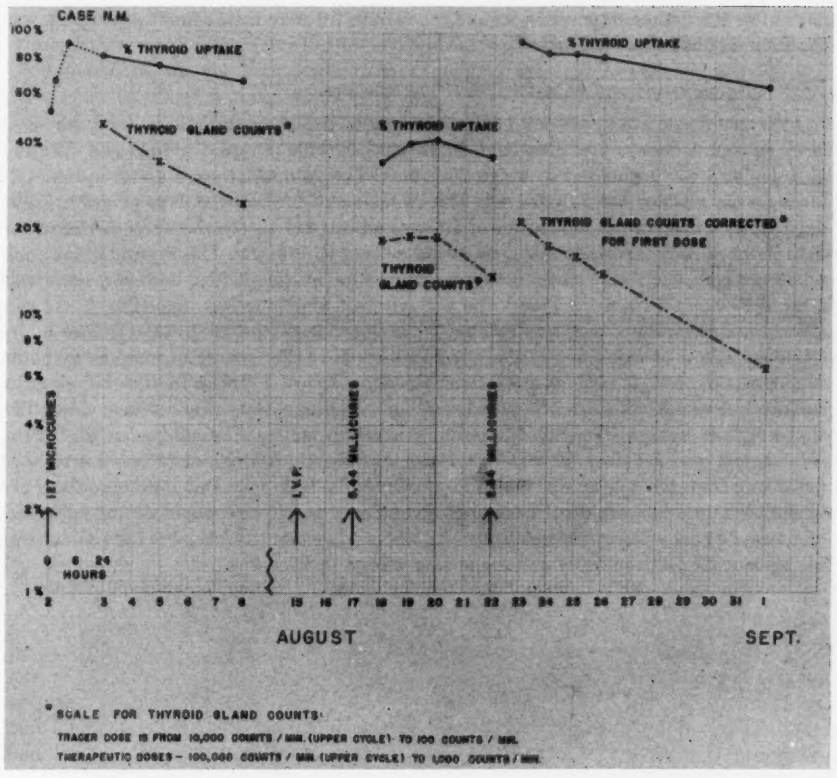
CASE N.M.
% THYROID UPTAKE
THYROID GLAND COUNTS*
% THYROID UPTAKE
% THYROID UPTAKE
THYROID GLAND COUNTS*
THYROID GLAND COUNTS CORRECTED* FOR FIRST DOSE
187 MICROCURIES
I.V.P.
6.44 MILLICURIES
6.44 MILLICURIES
HOURS
AUGUST
SEPT.
*SCALE FOR THYROID GLAND COUNTS:
TRACER DOSE IS FROM 10,000 COUNTS / MIN. (UPPER CYCLE) TO 100 COUNTS / MIN.
THERAPEUTIC DOSES – 100,000 COUNTS / MIN. (UPPER CYCLE) TO 1,000 COUNTS / MIN.

FIG. 2. Case 22, N.M. The influence of intravenous Diodrast on the thyroid gland uptake and turnover of I^{131}. (*Addendum.* The percentage uptakes of the second therapeutic dose have been inadvertently plotted 10 per cent higher than the actual values.)

days after the first dose. Eighty-four per cent of the second dose was taken up by the thyroid gland in twenty-four hours and the biologic half-life of the second dose was 5.0 days. It was estimated that 6,100 REP were delivered by the first dose and 3,900 REP by the second—a total of 10,000 REP.

During the next month the gland shrank in size and over the succeeding several months could no longer be palpated. The basal metabolic rate fell and six months after treatment was −6 per cent. The patient was asymptomatic.

Comment: The intravenous administration of Diodrast was followed by a marked decrease in the uptake of a therapeutic dose of I^{131} in comparison with the thyroid uptake of a tracer dose administered before the Diodrast. The I^{131} turnover was also considerably slower than that following the tracer dose. These effects upon the uptake and turnover of I^{131} following Diodrast persisted for approximately 120 hours. The consequent undertreatment was recognized and additional I^{131} was administered. It is of considerable interest that the uptake and turnover of a second therapeutic dose administered five

days after the first therapeutic dose and seven days after an intravenous pyelogram was the same as that observed after the tracer.

Effects of high iodine content of food on I^{131} metabolism

Case 26. F.K., a 33-year-old woman, presented the characteristic history, physical findings and laboratory evidence of thyrotoxicosis. The thyroid gland was diffusely enlarged and was estimated to weigh 50 grams. The 24-hour thyroid gland uptake following a tracer dose was 74 per cent. The biologic half-life was 5.0 days (Fig. 3). Eight days later, a dose of 6.9 millicuries of I^{131} was administered. On the basis of the tracer data it was anticipated that this dose would deliver 10,100 REP. The thyroid gland took up 69 per cent (4.8 millicuries) in twenty-four hours. Subsequently, however, there was a rapid loss of I^{131} from the gland, the biologic half-life being 3.1 days (Fig. 3). It was ascertained that the patient had eaten a "double serving" of scallops thirty-three hours following administration of the therapeutic dose. No other source of exogenous iodide intake was elicited. In order to correct for the rapid loss of I^{131} from the thyroid gland, a second dose of 4.2 millicuries of I^{131} was administered five days after the first dose. The thyroid gland uptake was 18 per cent in twenty-four hours; the biologic half-life of the second dose was 5.4 days. It was estimated that the thyroid gland received a total of 6,800 REP from both doses of I^{131}, 5,700 REP from the first dose and 1,100 REP from the second. There was a rapid subsidence of symptoms; the thyroid gland decreased in size and two months after treatment was not palpable. Six months after I^{131} the patient was asymptomatic and the basal metabolic rate was ± 0 per cent.

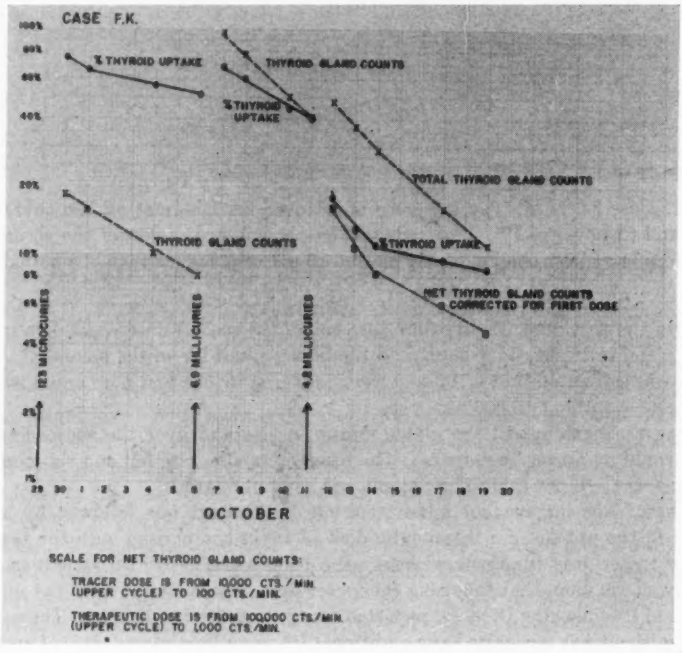
CASE F.K.
% THYROID UPTAKE
THYROID GLAND COUNTS
% THYROID UPTAKE
TOTAL THYROID GLAND COUNTS
THYROID GLAND COUNTS
% THYROID UPTAKE
NET THYROID GLAND COUNTS CORRECTED FOR FIRST DOSE
123 MICROCURIES
6.9 MILLICURIES
4.2 MILLICURIES
OCTOBER
SCALE FOR NET THYROID GLAND COUNTS:
TRACER DOSE IS FROM 10,000 CTS./MIN. (UPPER CYCLE) TO 100 CTS./MIN.
THERAPEUTIC DOSE IS FROM 100,000 CTS./MIN. (UPPER CYCLE) TO 1,000 CTS./MIN.

FIG. 3. Case 26, F.K. The effect of a high iodine meal on the uptake and turnover of a therapeutic dose of I^{131}.

Comment: The thyroid gland turnover of I^{131} following a therapeutic dose was 1.6 times more rapid than following the tracer dose. The 24-hour thyroid gland uptake, however, following the therapeutic dose was only 7 per cent less than the tracer dose. It is believed that the increased turnover was due to the ingestion of scallops, a sea food of moderate iodine content, thirty-three hours after the therapeutic dose. When the discrepancy between the anticipated and actual delivered radiation was recognized, a second dose was administered. The uptake of the second dose was markedly decreased. Since there was no change in the clinical status of this patient, the low uptake does not necessarily reflect diminished thyroid toxicity.

It is of interest to compare the results of the second therapeutic dose in this patient with the results in Case 22. In the latter instance the uptake of a second therapeutic dose was 84 per cent in contrast to an uptake of 35 per cent following the first therapeutic dose administered five days previously. In Case 26 the uptake of the second dose was only 18 per cent, in contrast to 69 per cent following the first dose also administered five days previously. The low initial uptake in Case 22 can be ascribed to the prior intravenous pyelogram. The low uptake of the second therapeutic dose in Case 26 similarly might be considered due to the prior exhibition of iodides. However, the possibility exists that the lower uptake of the second dose in Case 26 is due to the higher initial I^{131} radiation and is a direct effect of radiation on the thyroid gland. A marked reduction in uptake and a rapid turnover following second therapeutic doses of I^{131} have been observed in euthyroid cardiac patients given two therapeutic doses one week apart (25).

Inadvertent overdosage with I^{131}. Correction by administration of stable iodide

Case 35. BIH #90796, R.F., a 35-year-old woman, was referred by Dr. M. F. Lesses because of thyrotoxicosis of six months' duration. In August 1949, a dose of 5.6 millicuries of I^{131} was administered. In December 1949, although significant improvement had occurred, mild thyrotoxicosis persisted. She was nervous and jittery; the pulse rate was 100. The basal metabolic rate had fallen from +40 to +17 per cent. The thyroid gland had decreased in size from an estimated 50 grams to 30 grams. The 24-hour uptake of a tracer dose was 76 per cent, with a biologic half-life of 5.2 days (Fig. 4).

It was decided to administer an additional 5,000 REP, or 1.8 millicuries of I^{131}. Inadvertently 5.3 millicuries, or three times the intended dose, was administered. The thyroid gland uptake twenty-four hours after this dose was 72 per cent. Potassium iodide was therefore given as illustrated in Figure 4. This resulted in a rapid decrease in I^{131} content of the thyroid gland. Four hours after the initial dose, 10 per cent of the I^{131} content of the thyroid gland had been discharged. During the twenty-four hours following administration of stable iodide the loss of thyroid radiation was 25 per cent of the dose. The urinary I^{131} excretion was 15 per cent, as compared to 2 per cent of the tracer dose during a similar period. During the first two days, the therapeutic biologic half-life was 1.7 days as compared to 3.2 days in a comparable period following the tracer. During the sixteen days of the study the biologic half-life of the therapeutic dose was considerably shorter than following the tracer (Fig. 4). It was estimated that 6,100 REP were delivered, whereas approximately 13,200 REP would have been delivered if potassium iodide had not been administered and the therapeutic dose had been metabolized in the same manner as the tracer.

Four months after the second therapeutic dose the patient was asymptomatic; the thyroid gland was not palpable and the basal metabolic rate was −5 per cent. One year after treatment, this euthyroid status had been maintained.

Comment: The inadvertent administration of three times the intended dose of I^{131}, was

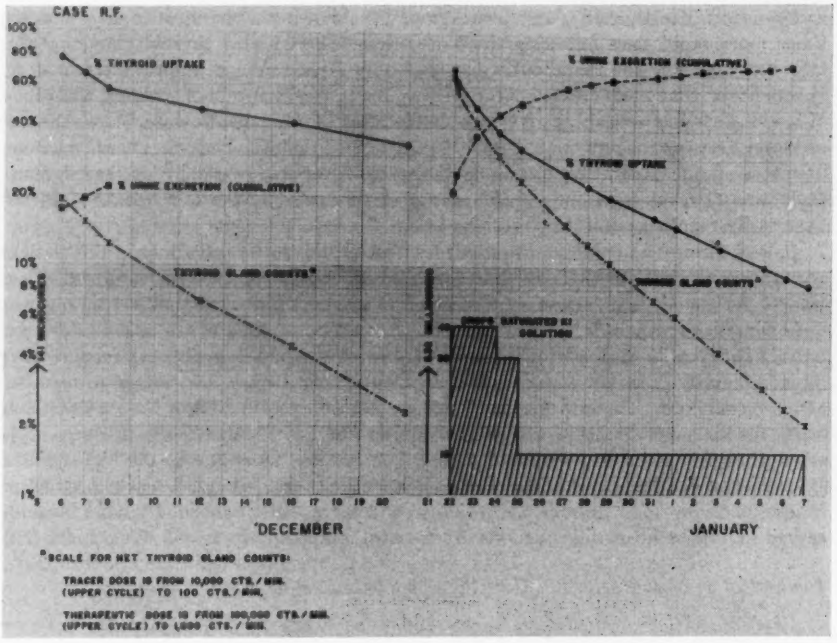

FIG. 4. Case 35, R.F. The effect of stable iodide on the biologic half-life of I^{131}. Administration of KI resulted in a prompt excretion of I^{131} and a consequent decrease in the radiation delivered to the thyroid gland.

corrected by the administration of stable iodide. It is of interest that the 24-hour thyroid uptake following the therapeutic dose was the same as that following the tracer. The administration of potassium iodide resulted in a prompt excretion of I^{131} and a consequent decrease in the radiation delivered to the thyroid gland. The therapeutic result was excellent.

The difficulty of estimating the biologic half-life in some cases

Case 31. BIH #M8951, B.H., a 32-year-old woman, was referred by Dr. S. L. Gargill for treatment of thyrotoxicosis of recent onset. The thyroid gland was diffusely enlarged and estimated to weigh 55 grams. The 24-hour thyroid uptake following the tracer dose was 84 per cent. The thyroid gland I^{131} content measured on five successive days showed an initially rapid turnover, estimated as a 3.6-day biologic half-life, followed by a markedly slower turnover (Fig. 5). The biologic half-life following a therapeutic dose of 6.1 millicuries of I^{131} was 6.5 days. The 24-hour thyroid gland uptake following the therapeutic dose was 76 per cent, or 10 per cent less than the tracer dose. It was estimated that the thyroid gland received 11,000 REP from the therapeutic dose. The result was excellent. Six months after therapy, the basal metabolic rate was +3 per cent and the patient was asymptomatic.

Comment: This case emphasizes the importance of obtaining sufficient data to estimate the biologic half-life, as has been pointed out by Werner and Quimby (9). If the

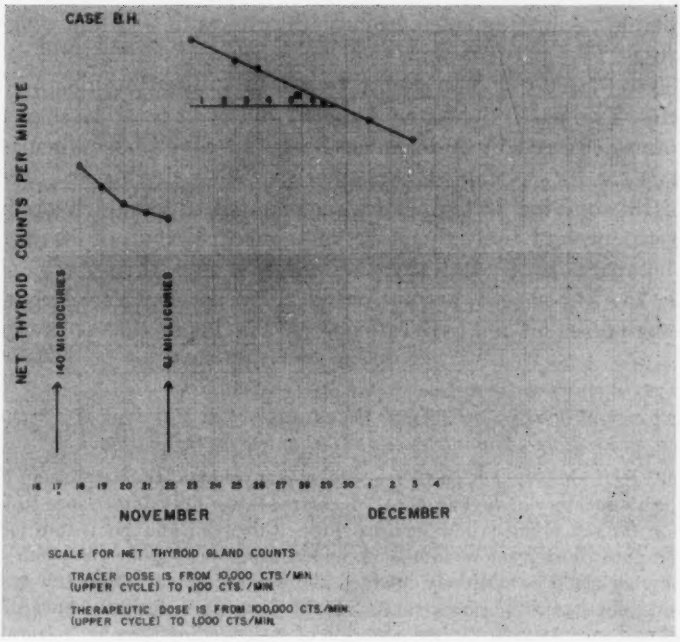
CASE B.H.
NET THYROID COUNTS PER MINUTE
140 MICROCURIES
9 MILLICURIES
NOVEMBER
DECEMBER
SCALE FOR NET THYROID GLAND COUNTS
TRACER DOSE IS FROM 10,000 CTS./MIN. (UPPER CYCLE) TO 1,100 CTS./MIN.
THERAPEUTIC DOSE IS FROM 100,000 CTS./MIN. (UPPER CYCLE) TO 1,000 CTS./MIN.

FIG. 5. Case 31, B.H. An illustration of the need for obtaining sufficient data to estimate the biologic half-life. The turnover of the tracer dose slowed notably after the third day.

data had been limited to those obtained the first three days after the tracer, 9 millicuries would have been administered. Despite the 10 per cent decrease in thyroid gland uptake, the thyroid radiation would have been 16,200 REP, or 47 per cent more than that actually delivered. According to the data presented subsequently, 16,000 REP is in the range of dosage in which myxedema might be anticipated.

Relationship of dose delivered to therapeutic effect

Sufficient data are available in 36 of the 42 patients to permit evaluation of the relationship between delivered radiation and the therapeutic effect (Table 2). Twenty-six of the 36 patients received one therapeutic dose of I^{131}; 20 were euthyroid after an estimated thyroid radiation varying from 5,800 to 17,000 REP (average 9,700). The remaining 6 patients receiving one dose became hypometabolic after a thyroid radiation of 8,000 to 19,100 REP (average, 10,900); 5 of the 6 received less than 10,000 REP. There was considerable overlap of dosage between these two groups (Table 2).

Ten of the 36 patients were not euthyroid or hypothyroid after an initial thyroid radiation ranging from 5,000 to 12,500 REP (average, 8,800); each patient, though improved, remained thyrotoxic. In 8 of the 10, data

are available permitting calculation of thyroid radiation following subsequent doses. Five patients were rendered euthyroid with a total radiation of 11,300 to 37,500 REP delivered in from 2 to 4 doses totalling 5.6 to 38.0 millicuries. Two patients became hypothyroid after total doses of 17,000–19,300 REP delivered by 2 doses totalling 12.3 and 15.3 millicuries. One patient (Case 12) remained persistently mildly thyrotoxic after three doses of I^{131} totalling 28.1 millicuries, estimated to have delivered 32,800 REP. At the present time, therefore, of the entire group of 34 patients in whom comparison of total thyroid radiation and therapeutic effect is possible, 25 (73 per cent) are euthyroid, 8 (24 per cent) are hypothyroid, and 1 has persistent mild thyrotoxicosis. The latter case is presented in detail.

Incomplete cure of thyrotoxicosis despite the administration of 3 doses of I^{131} totalling 28 millicuries, which delivered approximately 33,000 REP to the thyroid gland.

Case 12. BIH #M4508, J.K., a 42-year-old woman, entered the hospital on February 12, 1949, with a history of "eye swelling," increased fatigability, intolerance to heat, and palpitation for the previous few months. Propylthiouracil and potassium iodide, administered until shortly before admission, had not afforded significant relief.

The thyroid gland was diffusely enlarged and was estimated to weigh 90 grams. The basal metabolic rate was +50 per cent. Eight days after discontinuance of propylthiouracil and seventeen days after cessation of stable iodide therapy, a tracer dose of I^{131} was administered. The 24-hour thyroid gland uptake was 82 per cent and the biologic half-life was 4.3 days. Five days after the tracer, 12.8 millicuries of I^{131} was administered and 82 per cent was taken up by the thyroid gland in twenty-four hours, with a biologic half-life of 4.8 days. Potassium iodide, begun five days after the therapeutic dose, was omitted ten weeks later. Twelve weeks after I^{131} and two weeks after omission of potassium iodide, the patient, although improved, was still moderately thyrotoxic. The basal metabolic rate was +50 per cent; the thyroid gland was estimated to weigh 75 grams. An I^{131} tracer at this time showed an 80 per cent uptake in twenty-four hours with a biologic half-life of 5.0 days. Two weeks later, or four weeks after omission of stable iodide, 10 millicuries of I^{131} was administered and 83 per cent taken up in twenty-four hours, with a biologic half-life of 5.0 days. Five and one-half months after the first therapeutic dose and two months after the second therapeutic dose, the patient was still moderately thyrotoxic. The thyroid gland, however, was considerably reduced in size, and was estimated to weigh 45 grams. The basal metabolic rate was +50 per cent. The 24-hour tracer uptake at this time was 86 per cent and the biologic half-life was 4.6 days. Consequently a third therapeutic dose of 5.3 millicuries of I^{131} was administered, 77 per cent of which was taken up in twenty-four hours, with a biologic half-life of 6.0 days.

It was estimated that 11,000 REP had been delivered by the first dose, 11,000 by the second and 10,800 REP by the third, or a total of 32,800 REP. Eighteen months following the first therapeutic dose of I^{131} and twelve months after the third dose, the patient was still mildly thyrotoxic. The basal metabolic rate was +20 per cent, the 24-hour tracer uptake was 66 per cent, and the biologic half-life was 4.1 days. The thyroid gland was estimated to weigh 35 grams. Potassium iodide has recently been administered, with a consequent fall in the basal metabolic rate to +6 per cent and a significant clinical improvement.

Comment: This patient illustrates an unusual resistance to radioactive iodine. Three doses of I^{131} totalling 28 millicuries, estimated to have delivered approximately 33,000 REP, resulted in definite improvement; but eighteen months after the first dose and twelve months after the third dose, mild thyrotoxicosis persisted.

DISCUSSION

The present investigation was undertaken to ascertain the possibility of delivering a predetermined amount of radiation to the thyroid gland utilizing the metabolism of a tracer dose as a guide, and to delineate the relationship between the estimated I^{131} radiation delivered to the thyroid gland and the therapeutic effect.

The prediction of delivered radiation

It would appear from our data that it is possible to predict the uptake of a therapeutic dose on the basis of the tracer uptake. In 53 comparisons of tracer and therapeutic uptakes a difference greater than 20 per cent was noted in only 5; in 4 of these 5 instances the recent administration of a thiourea compound or iodide or iodine ingestion between the tracer and the therapeutic doses provided a satisfactory explanation for the observed differences in uptake. It is believed that the close agreement between tracer and therapeutic uptakes is not fortuitous. The method of direct measurement of thyroid uptake utilized in these studies has been experimentally demonstrated to have an accuracy of ± 5 per cent (2). Moreover, excellent agreement has been obtained in uptake studies following 2 to 4 closely repeated tracer doses in both euthyroid and thyrotoxic subjects (3). It is known, however, that variations in the clinical severity of thyrotoxicosis are frequently observed; the present data are, therefore, applicable only when tracer and therapeutic doses are administered at close intervals. Other authors (10) have noted excellent agreement between tracer and therapeutic uptakes, utilizing urinary I^{131} excretion as an indirect measure of thyroid uptake; detailed individual differences were not noted.

The prediction of the biologic half-life of a therapeutic dose from the biologic half-life of a tracer is not as reliable as the prediction of the 24-hour uptake. It has been stated that the rate of secretion of radioiodine from the thyroid gland cannot be anticipated with certainty even by *in vivo* studies of tracer doses because one of the early variable effects of a therapeutic dose is an increase in the rate at which radioiodine leaves the gland (10). Williams (14) stated that "even tracer dose technique did not reflect the alterations in iodine metabolism that occurred during the weeks that I^{131} exerted its activity." In our studies the average difference between the biologic half-life of the therapeutic dose and that of its corresponding tracer was only 11.5 per cent, comparing favorably with the

8.6 per cent difference found in uptake studies; however, individual discrepancies in biologic half-life could not be explained (Table 2). It should be also noted that although the discrepancies in biologic half-life between tracer and therapeutic doses were not consistently in the same direction, the biologic half-life of the therapeutic dose was usually less (Cases 3, 4, 8, 13, 30 and 37) than that of the tracer. This is consistent with the increased levels of serum protein-bound iodine occasionally found after I^{131} therapy (13, 26). It must be emphasized, however, that the present studies describe the early period (two weeks) following I^{131} administration, during which approximately 75 per cent of the radiation is delivered. In this period, in contrast to the statements in the literature, it is apparent from our studies that in most instances (75 per cent) the biologic half-life following therapeutic doses can be predicted from the biologic half-life following tracer doses.

Werner (18) found the biologic half-life following therapeutic doses of I^{131} in 103 thyrotoxic patients to vary between four and seven days, averaging six days. Others (15) have assumed the biologic half-life to be six days. Most clinics have made little use of this measurement. It is obvious, however, that the rate of loss of I^{131} from the thyroid gland is important in determining the total delivered radiation. Measurement of dosage, whether expressed as roentgen equivalents, as microcuries per gram, or as millicuries delivered, is inexact unless corrected for the rate of loss of I^{131}. The range of biologic half-life from 2.7 to 8 days, average 5.5 (S.D., 1.3) obtained by us in 53 cases of thyrotoxicosis makes obvious the error incurred in utilizing an average biologic half-life in calculating roentgens delivered or in assessing dosage on the basis of millicuries administered or even millicuries retained in the gland in twenty-four hours.

The data presented in this report indicate that the predictability of radiation delivered is sufficiently accurate to allow calculated I^{131} dosage. In attempting to provide 100 to 200 microcuries per estimated gram of tissue and thus deliver 5,000 REP, Werner noted that the "initial goal of dosage was only roughly achieved and an even wider range of radiation resulted" (9). In our present study, however, the percentage difference between the delivered REP and that anticipated on the basis of the tracer studies averaged only 16.8 per cent. In 19 of 50 comparisons (38 per cent) the difference between anticipated and delivered REP was less than 10 per cent. In the instances of marked discrepancies, as has been noted above, the source of the error was ascertained; in some cases this could have been prevented (Cases 22, 26, 35).

The relationship between administered radiation and therapeutic result

Table 1 summarizes the therapeutic results obtained in over 1,000 cases of thyrotoxicosis treated with I^{131}; the radiation delivered to the thyroid

gland was estimated in only 2 series. Werner *et al.*(9) noted an incidence of 50 per cent failure with a dosage under 5,000 REP; some instances of therapeutic failure were observed after the delivery of more than 10,000 REP. Feitelberg *et al.* (15) administered a dose of I^{131} calculated to deliver 10,000 equivalent roentgens; the biologic half-life was assumed to be six days. Data on the actual radiation delivered were not presented but good results were achieved in the vast majority of the 290 patients treated; the incidence of hypometabolism was only 6 per cent. In our series, we attempted to deliver 9,000 to 11,000 REP. In 20 of 26 patients treated with a single dose of 5,800 to 17,000 REP (average 9,700), a euthyroid state was

TABLE 3. THE RELATION BETWEEN THYROID GLAND WEIGHT, DELIVERED RADIATION AND CLINICAL EFFECT FOLLOWING A SINGLE THERAPEUTIC DOSE OF I^{131} IN 38 PATIENTS WITH THYROTOXICOSIS

	Status of patients following delivery of number of REP of I^{131} therapy noted below		
	Euthyroidism	Hypothyroidism	Persistent thyrotoxicosis
More than 75 grams	17,000 (1 patient)		5,000; 11,000; 6,900; 7,800 (4 patients)
50–75 grams	9,700; 6,500; 8,500; 6,800; 5,800; 7,500; 11,000; 12,000; 7,500 (9 patients)	8,300; 9,900; 19,000; 8,600; 8,000 (5 patients)	12,500; 7,400; 10,500; 8,000; 11,300; 8,000 (6 patients)
Less than 50 grams	9,700; 9,000; 9,200; 10,400; 6,100; 11,900; 10,000; 11,000; 6,100; 8,000; 10,800; 10,500 (12 patients)	9,500 (1 patient)	

achieved. In 6 of the 26 cases (20 per cent) hypometabolism was observed with initial doses of 8,000 to 19,100 REP (average, 10,900). In no case was hypometabolism observed with an initial dosage under 8,000 REP. In 1 patient (Case 18), however, with a thyroid gland estimated to weigh 100 grams, a single dose of 17,000 REP resulted in euthyroidism. Moreover, in an additional 10 cases similar initial doses of 5,000 to 12,500 REP (average, 8,800), although resulting in improvement, did not terminate the hyperthyroidism. This failure to correlate I^{131} dosage and therapeutic effectiveness completely may be consequent to several factors. 1) The REP delivered may have been incorrectly calculated due to inaccurate estimation of thyroid gland size. Estimation of the weight of the thyroid gland,

the third variable in the measurement of the amount of radiation delivered per gram of tissue is generally regarded to be in error by approximately 20 to 30 per cent or more (9, 13). The data presented in Table 3 are of considerable interest in this regard. It is apparent that the greatest incidence of hypothyroidism and a high incidence of persistent thyrotoxicosis are observed in the group of patients whose glands were estimated to be two to three times normal size (50 to 75 grams); and, the incidence of persistent thyrotoxicosis after I^{131} therapy is least in patients whose thyroid glands were estimated to weigh less than 50 grams. Furthermore, the only patient rendered euthyroid by a single dose in the small group of patients with a thyroid gland estimated to be larger than 75 grams, received 17,000 REP, well above the radiation delivered in the remaining 4 patients of this group with large glands. 2) Uneven distribution of I^{131} in the thyroid gland may result in non-uniform irradiation of the gland and persistence of non-irradiated tissue. Previous studies suggest that this may indeed be the case (27). A given dose may, therefore, have a variable effect. 3) It has been suggested that the biologic sensitivity of the thyroid epithelium may vary from patient to patient. 4) Finally, if the initial dose of I^{131} fails to induce a euthyroid state, the estimation of radiation consequent to subsequent doses is made difficult by uncertainty regarding the amount of residual functioning thyroid tissue as distinct from the amount of palpable thyroid tissue. It is, therefore, of interest that for some patients a second dose smaller than the first has been adequate to induce a euthyroid state. Other patients, however, have seemingly demonstrated remarkable resistance to the effects of repeated I^{131} radiation. Further studies are in progress to elucidate these various possibilities.

SUMMARY

1. This investigation was undertaken to ascertain 1) whether the thyroid uptake and turnover (biologic half-life) following a therapeutic dose of I^{131} was predictable from the uptake and turnover following a tracer dose, and 2) the relationship between the estimated radiation delivered by the therapeutic dose of I^{131} and the therapeutic effect. The method utilized for measurement of thyroid uptake and turnover by external counting consisted of four Geiger-Mueller tubes connected in a parallel electrical circuit and arranged in a circle of 45 cm. radius in a horizontal plane about the neck. This method provides quantitative measurement of I^{131} thyroid gland content, virtually independent of the size and location of the thyroid gland.

2. In 53 comparisons of tracer and therapeutic doses in 42 patients with thyrotoxicosis, the average uptake of the tracer dose twenty-four hours after I^{131} administration was 72.7 per cent (S.E., 1.8 per cent) and the aver-

age uptake of the therapeutic dose was 72.4 per cent (S.E., 2.4 per cent). The average difference between the 24-hour uptake measured after a therapeutic dose and that measured after the tracer dose in the same patient was 8.6 per cent (S.E., 1.7 per cent).

In 5 of the 53 comparisons the uptake of the therapeutic dose differed from that of the tracer dose by 20 per cent or more. In 4 of these 5 instances the probable source of the discrepancy was either the recent omission or the administration of stable iodide (potassium iodide and organic iodo compounds) or of antithyroid drugs between the tracer and therapeutic doses.

3. The average biologic half-life following the tracer dose was 5.5 days (S.E., 1.7) and following the therapeutic dose, 5.5 days (S.E., 1.5). The average difference between the biologic half-life measured after the therapeutic dose and that found after the tracer dose in the same patient was 11.5 per cent (S.E., 1.4 per cent).

In 11 instances in 10 patients the difference between the biologic half-life of a therapeutic dose and that of its corresponding tracer dose was 20 per cent or greater. In 8 of the 11 the biologic half-life of the therapeutic dose was shorter than that of the corresponding tracer dose; in the remaining 3 it was longer. In 1 case, the difference was due to the administration of Diodrast between the tracer and therapeutic dose; no adequate explanation is available for the differences observed in the other cases.

4. The predictability of the delivered thyroid radiation of a therapeutic dose, on the basis of the uptake and biologic half-life following a tracer dose is sufficiently accurate to allow for calculated I^{131} dosage. In 50 comparisons made in 40 patients the REP delivered by the therapeutic dose to the thyroid gland differed from that anticipated on the basis of the tracer studies by an average of 16.8 per cent (S.E., 1.8 per cent).

5. Evaluation of the relationship between delivered radiation and therapeutic effect was possible in 36 of the 42 patients. Twenty-six of the 36 patients received only one therapeutic dose of I^{131} ranging from 2.7 to 25.0 millicuries; 20 patients were euthyroid after an estimated thyroid radiation of 5,800 to 17,000 REP (average, 9,700). The remaining 6 patients receiving one dose became hypometabolic after a thyroid radiation of 8,000 to 19,100 REP (average, 10,900); 5 of the 6 received less than 10,000 REP. There was considerable overlap of dosage in these two groups.

Ten of the 36 patients, though improved, remained thyrotoxic after an initial thyroid radiation ranging from 5,000 to 12,500 REP (average, 8,800). Five of the 10 have been rendered euthyroid with a total radiation of 11,300 to 37,500 REP delivered in two to four doses totalling 5.6 to 38.0 millicuries. Two have become hypothyroid after a total dosage of 17,000 to 19,300 REP delivered by two doses totalling 12.3 and 5.3 millicuries. One

patient has remained persistently mildly thyrotoxic after three doses of I^{131} totalling 28.1 millicuries, estimated to have delivered 32,800 REP. Thus, of the 34 patients in whom comparison of total thyroid radiation and therapeutic effect was possible 25 (73 per cent) are euthyroid, 8 (24 per cent) are hypothyroid, and 1 presents persistent mild thyrotoxicosis. The greatest incidence of hypothyroidism and a high incidence of persistent thyrotoxicosis was observed in the group of patients whose glands were estimated to be two to three times (50–75 grams) normal size. The incidence of persistent thyrotoxicosis or myxedema after administration of I^{131} was least in patients whose thyroid glands were estimated to weigh less than 50 grams.

CONCLUSIONS

The thyroid uptake and turnover (biologic half-life) of a therapeutic dose of I^{131} can be determined from the uptake and turnover following a tracer dose.

The predictability of the delivered thyroid radiation of a therapeutic dose of I^{131}, on the basis of the uptake and biologic half-life following a tracer dose, is sufficiently accurate to allow for calculated I^{131} dosage.

Addendum

A recent paper has come to our attention (Blomfield, G. W., *et al.*: Treatment of thyrotoxicosis with radioactive iodine, *Brit. M. J.* **2**: 373–381 (Aug. 18), 1951). These investigators undertook a comparison of thyroid uptake and biologic half-life following tracer and therapeutic doses of I^{131} in 20 patients with thyrotoxicosis. Their conclusions are in agreement with those reported by us.

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THE TREATMENT OF NODULAR GOITER

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THE clinical course of nodular goiter is varied. The thyroid nodule may remain unchanged for long periods of time; it may grow and exert pressure on neighboring structures; it may degenerate or be the site of hemorrhage; it may be neoplastic and infiltrate adjacent tissues or metastasize; rarely it may hyperfunction within itself, or, more commonly, be associated with perinodular hyperfunction; and, very infrequently, it will spontaneously disappear.

Single or multiple nodules which produce pressure or which become associated with thyrotoxicosis are readily treated by thyroidectomy. Those nodules which are suspected of malignancy because of recent growth or unusual firmness are similarly best treated by thyroidectomy. The treatment of single or multiple nodules which are entirely asymptomatic remains controversial. The purpose of this report is to present an analysis of our experience in the treatment of nodular goiter, and a discussion of its significance in the early diagnosis and treatment of thyroid cancer.

MATERIAL

This study comprises data on 200 patients with nodular goiter, all of whom were operated upon by the surgeons of the Ward Surgical Service and followed in the Endocrine Clinic. There were 182 females and 18 males, a ratio of 10:1. The ages ranged from the first to the eighth decade. All patients in this series had an evaluation of thyroid function by clinical examination, basal metabolism tests, and blood cholesterol determinations. Roentgenograms of the chest, and vocal cord examinations were made in every case.

INDICATIONS FOR OPERATION

The indications for operation are listed in Table 1. Nine patients had more than one operation, so that the total number of operations was 210. In 67 patients the operation was performed solely because of the palpation of a solitary nodule, unassociated with symptoms. Ninety-one patients had noted recent growth of the thyroid nodule or nodules, or pressure symptoms. In 33 patients, the nodules were associated with thyrotoxicosis. Six patients had been operated upon elsewhere, and were reoperated upon

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TABLE 1. INDICATIONS FOR OPERATION

	Number of cases
1. Nodule alone	67
2. Nodule with growth or pressure symptoms	91
3. Nodule with thyrotoxicosis	33
4. Recurrence of nontoxic nodule	
a. Previous operation elsewhere	6
b. Previous operation at Beth Israel Hospital	8
5. Recurrence of nodule with thyrotoxicosis	
a. Previous operation elsewhere	3
b. Previous operation at Beth Israel Hospital	2
Total	210

because of recent thyrotoxicosis associated with a recurrent nodule. Eight patients previously operated upon at the Beth Israel Hospital required reoperation because of the recurrence of nontoxic nodular goiter. Two patients previously operated upon at the Beth Israel Hospital had reoperations for recurrent toxic nodular goiter. The types of operations are shown in Table 2.

TABLE 2. TYPES OF OPERATION

200 patients	210 operations
1. Excision of nodules (single or multiple)	72
2. Unilateral subtotal thyroidectomy	48
3. Unilateral subtotal thyroidectomy and excision of isthmus or of nodule in opposite lobe	7
4. Bilateral subtotal thyroidectomy	51
5. Unilateral total hemithyroidectomy	28
6. Bilateral total thyroidectomy	2
7. Biopsy	1
8. Excision of glands	1
Total	210

PATHOLOGIC DIAGNOSIS

The pathologic findings in each case are shown in Table 3. These may be divided into three categories: 1) non-neoplastic nodules, 2) benign neoplasms and 3) malignancy of the thyroid. In the first category the nodules were chiefly colloid in nature, associated with hyperplasia and in-

TABLE 3. PATHOLOGIC DIAGNOSES

	Number of cases
1. *Nodular Goiter (non-neoplastic)*	
a. Colloid nodule	112
b. Degenerated cyst	6
c. Fetal adenoma	23
d. Organizing hematoma	1
e. Fetal adenoma with degeneration	9
f. Hyperplasia	10
g. Hashimoto's struma	3
h. Chronic thyroiditis	1
Total	166
2. *Benign Neoplasms*	
a. Embryonal adenoma	11
b. Fetal and embryonal adenomas	4
c. Papillary adenoma	2
Total	17
Total thyroids with non-malignant nodules	183
Total thyroids with malignant nodules (2 recurrent)	27
Total	210*

* Of the 200 patients, 8 were operated upon twice and 1, three times, making 210 specimens available for analysis.

volution in about 50 per cent of the cases. About one sixth of the patients had fetal adenomas, some of which were degenerating. Finally there were 3 instances of Hashimoto's struma and 1 case of chronic thyroiditis.

Benign neoplasms constituted 8 per cent of the entire series. These consisted chiefly of embryonal adenomas with 2 instances of papillary adenoma. These tumors were considered benign histologically since they were entirely intracapsular, showed no blood vessel invasion, and had not metastasized. Clinically their behavior was benign.

Malignant tumors of the thyroid were present in 25 cases (12.5 per cent).

PATHOLOGIC DIAGNOSIS OF THE MALIGNANT NEOPLASMS

Of the patients with solitary nodules, as determined pathologically, 15 or 14.4 per cent had cancer of the thyroid. The pathologic evidence for carcinoma depended upon the finding of blood vessel invasion in 5 patients, embryonal adenomas in 4, and fetal adenoma in 1. There were 7 cases of

papillary carcinoma, and 1 each of scirrhous carcinoma, epidermoid carcinoma, and giant cell carcinoma. Of the patients with multiple nodules, 10 or 10.4 per cent had malignancy of the thyroid. In this group, 1 had an embryonal adenoma with blood vessel invasion, 8 had papillary carcinoma, and 1 had diffuse small-cell carcinoma (Table 4). Invasion beyond the capsule of the thyroid into the muscle and regional lymph nodes was found in 6 cases or in 24 per cent of the 25 patients with thyroid

TABLE 4. PATHOLOGY AND INCIDENCE OF CANCER IN THYROIDS WITH SINGLE AND MULTIPLE NODULES

Pathologic diagnosis	Number of cases with	
	Single nodule	Multiple nodules
Embryonal adenoma with blood vessel invasion	4	1
Fetal adenoma with blood vessel invasion	1	
Papillary carcinoma	7	8
Epidermoid carcinoma	1	
Scirrhous carcinoma	1	
Giant cell carcinoma	1	
Diffuse small-cell carcinoma		1
Total	15	10
Total cases	25	
The incidence* of cancer of the thyroid in 200 cases of nodular goiter		12.5%
The incidence of cancer in single nodules		14.4%
The incidence of cancer in thyroids with multiple nodules		10.4%

* The incidence is calculated from the number of patients and not from the number of nodules.

cancer. Thus, in almost one fourth of the cases diagnosed as cancer there was malignancy in behavior as well as in appearance, as established by transcapsular invasion.

CLINICAL EVALUATION OF THYROID NODULARITY AS COMPARED WITH PATHOLOGIC EXAMINATION

In 190 patients it was possible to compare the clinical estimate of nodularity with the pathologic examination. One hundred and thirty-seven patients had solitary nodules on clinical examination. Forty-three of these proved to have multiple nodules on pathologic examination (Table 5). In 35 patients, multiple nodules were found both clinically and pathologically. In 18 patients with clinical diffuse enlargement of the thyroid, pathologic

TABLE 5. CLINICAL EVALUATION OF THYROID NODULARITY AS COMPARED WITH EXAMINATIONS OF EXCISED SPECIMENS

Clinical diagnosis	No. of cases	Pathologic examination	No. of cases
Single nodules	137	Single nodules	94
		Multiple nodules	43
Multiple nodules	35	Multiple nodules	35
*Thyroid diffusely enlarged (non-nodular)	18	Single nodules	5
		Multiple nodules	13
Total	190	Total	190

* In these thyroids the nodules were discovered only on examination of the excised specimen.

examination disclosed 5 cases with single nodules and 13 with multiple nodules. Thus the number of thyroids with solitary nodules as determined on the excised specimen was 94 in contrast to 137 found clinically; the remaining 43 glands showed multiple nodules.

The total number of patients with multiple nodules found by pathologic examination was 91, as contrasted with 34 diagnosed clinically. Thus multiple nodules of the thyroid are frequently overlooked, appearing either as diffuse enlargements of the entire gland or as a single nodular mass.

TREATMENT

All patients were evaluated in the Endocrine Clinic prior to thyroidectomy. The state of thyroid function, the degree of pressure upon the trachea and esophagus, the mobility of the vocal cords, and the extent of substernal projection were determined by measurement of the basal metabolism, the concentration of cholesterol in the serum, the determination of the serum precipitable iodine in several of the cases, direct laryngoscopy, and roentgenograms of the chest. The operations were performed in approximately half the cases by the surgical members of the Endocrine Clinic and the remainder by staff surgeons or the resident staff.

Thyroidectomy was performed through a low collar incision, and the strap muscles were sectioned, particularly in those cases in which the goiter was large. The recurrent laryngeal nerves were identified only when the extent of the goiter involved dissection along the usual course of these nerves. A search for the parathyroids was undertaken only with total lobectomy or total thyroidectomy. Postoperatively, all patients were examined for evidence of recurrent laryngeal nerve damage, hypopara-

thyroidism, hypothyroidism, persistent or recurrent thyrotoxicosis, and recurrent nodules.

Patients with toxic nodular goiter were prepared for thyroidectomy with saturated solution of potassium iodide in daily doses of 10 drops for periods of ten days to three weeks. In the 33 patients with toxic nodular goiter, excision of the nodule alone was carried out in 1 case, subtotal hemithyroidectomy in 2 cases, total hemithyroidectomy in 2 cases, and bilateral subtotal thyroidectomy in the remaining 28 cases. One case of persistent thyrotoxicosis was observed following subtotal lobectomy; in 2 cases a toxic nodular goiter recurred after bilateral subtotal thyroidectomy, and 4 cases of permanent myxedema developed following bilateral subtotal thyroidectomy. No instance of hypoparathyroidism or of injury to the recurrent laryngeal nerves occurred in this group of patients.

TABLE 6. COMPLICATIONS

	Number of cases
1. Recurrent nerve paralysis	
a. Unilateral	
Following excision of nodules only	7
Following unilateral subtotal thyroidectomy	2
Following unilateral hemithyroidectomy	4
Following bilateral subtotal thyroidectomy	3
Following bilateral total thyroidectomy	1
Total	17 (8.1%)*
Permanent	15 (7.15%)
Transient	2 (0.95%)
b. Bilateral	
Following bilateral subtotal thyroidectomy	2 (0.95%)
Permanent	0
Transient	4 (1.9%)
2. Postoperative hemorrhage	1 (0.5%)
3. Pulmonary atelectasis	2 (0.9%)
4. Wound sepsis	4 (1.9%)
5. Hematoma of wound	3 (1.4%)
6. Tetany	4 (1.9%)
a. Transient	3 (1.4%)
b. Permanent	1 (0.5%)
7. Myxedema (permanent)	13 (6.2%)
Mortality	1 (0.5%)

* All percentages are calculated from the total number of operations.

In 91 patients, thyroidectomy was carried out because of recent growth of the goiter or of pressure symptoms. Most of these patients had large multinodular goiters which not only compressed and deviated the trachea, but also extended into the retromanubrial space or occasionally behind the trachea or esophagus. The operations in this group, therefore, usually consisted of bilateral subtotal thyroidectomy. It is of interest that the incidence of injury to the recurrent laryngeal nerves was lower with this operation than in those patients with a large nodule involving a single lobe and requiring only unilateral thyroidectomy (Table 6).

Asymptomatic solitary nodules were operated upon in 67 patients. The usual methods of preoperative and postoperative study were employed in this group of cases. The majority of these patients required only unilateral procedures. In spite of this, injury to the recurrent laryngeal nerve was significantly greater than after bilateral subtotal thyroidectomy.

The patients with recurrent toxic and nontoxic nodular goiter, totalling 19, had had one or more previous thyroidectomies. The methods of preoperative preparation and postoperative follow-up were the same as in the primary cases of toxic and nontoxic nodular goiter. In this group, every effort was made to identify and preserve the parathyroids and to demonstrate the course of the recurrent laryngeal nerves. The recurrent nodule frequently appeared in the lobe that had not been operated on, so that it may have been unrecognized at the time of the first operation.

RESULTS IN PATIENTS WITH MALIGNANT NEOPLASMS

Twenty-five cases of cancer of the thyroid occurred in the 200 patients with nodular goiter. These patients ranged in age from 12 to 73 years, with 4 males and 21 females. Only 1 patient (Case 24) presented unequivocal clinical signs of thyroid cancer, namely unusual hardness of the mass, recurrent laryngeal nerve involvement, fixation to adjacent structures, and regional lymph node enlargement.

The incidence of cancer in the entire series of patients was 12.5 per cent; 14.4 per cent in those with proved solitary nodules, and 10.4 per cent in the group with proved multiple nodules. Papillary carcinoma was the most frequently encountered neoplasm, occurring in 15 of the 25 cases of malignancy. Embryonal adenoma with blood vessel invasion occurred in 5 cases, and the remainder was made up of single instances of fetal adenoma with blood vessel invasion, epidermoid carcinoma, scirrhous carcinoma, giant cell carcinoma, and diffuse small-cell carcinoma. The papillary carcinomas were distinguished from the papillary adenomas by the following histologic criteria of malignancy: 1) pleomorphism, 2) mitoses, 3) abnormal staining characteristics, and 4) invasiveness. In the embryonal adenomas and the fetal adenomas with blood vessel invasion, the

Table 7. Cancer of the thyroid

Patient				Pathologic findings in thyroid	Treatment	Follow-up
No.	Initials	Sex	Age			
1	T.G.	F	35	Embryonal adenoma with blood vessel invasion	Excision and postoperative irradiation	9 Years. Living and well. Permanent hypoparathyroidism and myxedema, adequately controlled by vitamin D and desiccated thyroid
2	M.N.	F	59	Embryonal adenoma with blood vessel invasion	Left lobectomy and excision of isthmus	No follow-up
3	S.G.	F	39	Papillary carcinoma	Total lobectomy and postoperative irradiation	9½ Years. No recurrence.
4	E.S.	F	21	Papillary carcinoma with lymph node invasion	Left total and right subtotal lobectomy, and postoperative irradiation	5 Years. No recurrence.
5	M.G.	F	34	Papillary carcinoma	Unilateral subtotal thyroidectomy	8½ Years. No recurrence.
6	A.K.	F	50	Embryonal adenoma with blood vessel invasion	Excision of nodule	11 Years. No recurrence.
7	J.O.	M	65	Epidermoid carcinoma	Left subtotal thyroidectomy, postoperative irradiation	15 Months. Died of diffuse metastases.
8	M.C.	F	20	Papillary carcinoma	Excision of nodules; postoperative irradiation	1 Year. No recurrence.
9	M.V.	F	32	Fetal adenoma with blood vessel invasion	Excision of nodule	16 Months. No recurrence.
10	E.S.	F	51	Papillary carcinoma	Excision of nodules; postoperative irradiation	11 Years. No recurrence.
11	A.D.	F	65	Papillary carcinoma	Incomplete excision of nodule; postoperative irradiation	5 Years. No recurrence.
12	S.S.	F	32	Papillary carcinoma with metastases to cervical glands	Right subtotal thyroidectomy; postoperative irradiatiou	5½ Years. No recurrence.
13	D.T.	F	42	Papillary carcinoma	Right hemithyroidectomy; postoperative irradiation	8 Years. No recurrence.
14	C.H.	F	55	Papillary carcinoma	Hemithyroidectomy; postopera- irradiation	10 Years. No recurrence. Myxedema controlled with thyroid.
15	I.C.	F	43	Papillary carcinoma with invasion of muscle	Bilateral subtotal thyroidectomy; postoperative irradiation	8 Years. No recurrence.
16	A.G.	M	62	Embryonal adenoma with blood vessel invasion	Right lobectomy	4 Years. No recurrence.
17	S.S.	M	12	Papillary carcinoma	Right lobectomy and excision of isthmus; postoperative irradiation	4 Years. Metastatic nodule in right lung, treated with radioactive iodine; no recurrence after 2 years.
18	L.M.	F	47	Papillary carcinoma with invasion into muscle	Right subtotal thyroidectomy; postoperative irradiation	1 Year. No recurrence.
19	F.S.	F	22	Papillary carcinoma metastatic to cervical glands	Excision of glands of neck; in one month right subtotal thyroidectomy with local excision of glands of neck; postoperative irradiation	8 Years. No recurrence.
20	F.C.	F	48	Papillary carcinoma	Bilateral subtotal thyroidectomy; postoperative irradiation	15 Years. No recurrence.
21	B.F.	F	20	Scirrhous carcinoma	Excision of nodule; postoperative irradiation	17 Years. Nodule recurred after 15 years. Re-operation—total thyroidectomy. Adenocarcinoma found, followed for 2 years since operation, with no recurrence.
22	B.K.	F	58	Papillary carcinoma	Excision of nodule; after 1 year, recurrent nodule (carcinoma). Total lobectomy; postoperative irradiation	13 Years. Myxedema. No recurrence.
23	M.L.	F	39	Embryonal adenoma with blood vessel invasion	Excision of nodules; postoperative irradiation	13 Years. Recurrent nodule. Re-operation—Hürthle cell adenoma. ? Malignant. No recurrence after 2½ years.
24	I.B.	M	73	Giant cell carcinoma	Biopsy	Died on fourteenth postoperative day.
25	B.C.	F	58	Diffuse small-cell carcinoma	Left hemithyroidectomy; postoperative irradiation	6 Years. Myxedema. No recurrence.

histologic criteria of malignancy consisted of: 1) evidence of thrombosis in the involved vessel, of longer duration than could be incident to operative trauma, 2) adherence of thyroid epithelial cells to the vessel wall, and 3) visualization of thyroid cells traversing the vessel walls.

The clinical characteristics of the cancerous and noncancerous nodular goiters in these patients did not differ, except for the patient mentioned previously, whose nodular goiter showed fixation and unusual irregularity and hardness. In many cases, the nodule or nodules appeared benign at operation, so that the final diagnosis of malignancy depended entirely upon the microscopic examination.

As may be seen in Table 7, the type of operation performed consisted of excision of a nodule or nodules in 8 cases, unilateral lobectomy in 6 cases, unilateral subtotal thyroidectomy in 8 cases, bilateral subtotal thyroidectomy in 2 cases, and biopsy alone in 1 patient (Case 24). In 1 patient (Case 11), incomplete excision of a carcinomatous nodule involving both lobes was performed because cord paralysis was discovered during operation. This patient has had no recurrence after five years. In 1 patient (Case 19) excision of involved cervical glands disclosed metastatic cancer, the thyroid origin of which was established at subsequent subtotal lobectomy. This patient has had no recurrence after eight years.

Postoperatively, 20 patients received external radiation of the thyroid region in doses of from 4,000 to 8,000 roentgens. In 4 patients, radiation was not used because of adequate excision of the neoplasm, and in 1 instance because the patient did not return. In these 4 patients, there has been no recurrence after eleven, eight and one-half, four, and one and one-third years, respectively. Except for 1 patient who died of heart disease fourteen days postoperatively, 23 of the patients were followed for from one to fifteen years postoperatively. One patient (Case 7) with epidermoid carcinoma died of diffuse metastases fifteen months postoperatively. One patient (Case 21) with scirrhous carcinoma at the age of 20, treated by excision of the nodule and intensive external radiation, showed a recurrence of cancer fifteen years later, as proved at secondary total thyroidectomy. This patient is living and well two years later, but did have postoperative hypoparathyroidism and hypothyroidism.

One patient (Case 17) with papillary carcinoma appearing at the age of 12, treated by total lobectomy and external radiation, had a metastatic nodule in the right lung one and one-half years later. This metastatic nodule proved to have thyroid function as shown by its uptake of radioactive iodine. It was treated successfully by means of a large dose of I^{131}, with disappearance of the metastasis and development of complete myxedema. This case has been reported elsewhere (1).

One patient (Case 22) with papillary carcinoma appearing at age 58 was

treated by excision of the nodule and external radiation. Another nodule appeared one year later in the opposite lobe. On reoperation the nodule proved also to be a papillary carcinoma adherent to the trachea. Total lobectomy was performed at this time and another course of roentgen therapy was given. Recurrent laryngeal nerve injury and myxedema developed postoperatively, but the patient is living and free of recurrence thirteen years later.

One patient (Case 23), with embryonal adenoma with blood vessel invasion appearing at age 39, was treated by excision of nodules and postoperative roentgen therapy. Thirteen years later another nodule appeared, which at operation proved to be a Hürthle cell adenoma of border-line malignancy. This patient has had no recurrence after two and one-third years.

One patient (Case 1) in this group had three thyroidectomies, the first two for control of Graves' disease associated with a diffusely enlarged hyperplastic goiter. The third thyroidectomy was necessitated by a recurrent nontoxic nodule which proved to be an embryonal adenoma of an angioinvasive type. The nodule was excised and postoperative external radiation of the thyroid was given. Following the operation hypothyroidism, hypoparathyroidism, and permanent unilateral vocal cord paralysis developed. These complications have been successfully controlled and the patient is entirely well nine years after the last operation.

In 2 other patients, 1 (Case 22) with papillary carcinoma and the other (Case 25) with diffuse small-cell carcinoma, myxedema developed postoperatively and was controlled with desiccated thyroid. They are otherwise well thirteen and five and one-half years, respectively, after operation.

DISCUSSION

The data presented in this paper indicate that patients with nontoxic nodular goiter require surgical exploration even when asymptomatic, because of the high incidence of malignancy in thyroid nodules. This point of view is not universally accepted, although numerous recent papers emphasize the association between nodular goiter and thyroid cancer. The reasons for the lack of general recognition of this association are twofold: 1) The discrepancy between the incidence of thyroid cancer in surgical specimens and in autopsy material, and 2) the lack of sufficient knowledge of the malignant potentialities of thyroid nodules. A discussion of these two factors, therefore, is pertinent.

Recent studies of the incidence of thyroid malignancy in routine autopsies have stressed the low incidence of thyroid cancer as a cause of death. Thus, VanderLaan (2) found only 5 thyroid cancers in 18,668

autopsies at the Boston City Hospital. At the Peter Bent Brigham Hospital and at the Massachusetts General Hospital, cancer of the thyroid gland caused death in less than 1 in 1,000 autopsied cases. Rogers, Asper, and Williams (3) have also found that carcinoma of the thyroid is rare in general hospitals. Among 544,918 admissions to three general hospitals, goiters occurred in 3,221 cases (0.59 per cent). A pathologic diagnosis of malignancy of the thyroid was made in 64 cases, or in 1.99 per cent of all patients with goiter. However, as pointed out by Anglem and Bradford (4), this series includes 907 patients with toxic diffuse goiter, 400 with nontoxic diffuse goiter, and 377 with toxic nodular goiter. In these forms of goiter the incidence of malignancy is very low. Thus, only 5 of the 64 cases of cancer were in the thyrotoxic group. When corrected, the incidence of carcinoma of the thyroid in patients with nontoxic nodular goiter in the series reported by Rogers, Asper, and Williams was 4.79 per cent for the entire series, and 11.2 per cent for the material they report from the Johns Hopkins Hospital (3). Anglem and Bradford (4), in a review of 1,657 autopsy records, found that cancer of the thyroid was not the cause of death in a single case, but neither did they find a single case of cancer of the tongue, floor of the mouth, gingiva, buccal mucosa, or palate, which as a group are more common than thyroid cancer. In a period shorter by two years than that covered by the autopsy survey, these authors found 11 patients who had died of thyroid cancer; only 1 of these came to autopsy and at another hospital.

According to Zimmerman (5), the concept of the etiologic relation of nodular goiter to thyroid cancer is based on three types of statistical evidence: i) the higher incidence of thyroid cancer in postmortem examinations in goitrous than in nongoitrous regions, paralleling the severity of goiter endemicity (6); ii) the high incidence of pre-existent nodular goiter in patients with cancer of the thyroid (7, 8, 9, 10); and iii) the frequency of cancer in surgically removed nodules of the thyroid.

There are many studies indicating pre-existent thyroid nodules in patients with thyroid cancer. Lahey, Hare, and Warren (7) found that 80 to 90 per cent of patients with malignant thyroid tumors had a history of pre-existent thyroid nodules. Horn and his co-workers (8) reported a similarly high incidence of thyroid cancer with a history of pre-existent nodular goiter. In 112 cases of carcinoma of the thyroid, Ward and his associates (9) noted that 77 per cent of the patients had a goiter prior to the development of symptoms suggestive of thyroid cancer.

The percentage of cancer of the thyroid in surgically removed nodules has varied considerably. Thus, in the Cleveland Clinic series, carcinoma was found in 3.4 per cent of multinodular goiters and in 24.5 per cent of solitary nodules of the thyroid (11). Anglem and Bradford (4) found that

7.6 per cent of 550 cases of nodular goiter were malignant. Cole and his associates (12) encountered an incidence of cancer of 17.2 per cent in nontoxic nodular goiters but 24 per cent of solitary nodules were malignant as contrasted with 9.8 per cent of malignancy in the multinodular group. Only 1 per cent of malignancy was encountered in patients with toxic nodular goiter. The experience of Cope and his co-workers (13) is in accord with that reported by Cole. Beginning in 1944, this group operated upon 156 patients with a single nodule or "unilateral abnormality of a single area of the thyroid," and found malignancy in 19 per cent. Pemberton and Black (14) found 18 carcinomas of the thyroid in 53 children under the age of 15 who had thyroidectomies for nontoxic nodular goiter. A high incidence of cancer in thyroid nodules is not unusual in children (15).

The etiologic relationship between pre-existing benign nodules and the development of thyroid carcinoma is difficult to establish in spite of the incidence of malignancy in surgically removed thyroid nodules. There is a considerable discrepancy in the frequency of cancer in excised nodules of the thyroid reported in the current and earlier literature (11). Zimmerman (5) has pointed out that in the period of intense goiter endemicity before the institution of goiter prophylaxis, the incidence of cancer in several large series of nodular goiters ranged from 1.6 to 2.8 per cent. With a sharp reduction in the prevalence of endemic goiter and nodular thyroid enlargements, there has been an apparently progressive increase in the percentage of thyroid carcinomas encountered in thyroid nodules. The probable explanation of this paradox may be that the frequency of thyroid cancer has remained constant, while that of non-neoplastic nodules has greatly diminished, with a resulting higher precentage of malignant tumors in the more recent series of thyroidectomies.

The validity of the histologic diagnosis of thyroid cancer is sometimes questioned because the postoperative survival rate of patients with thyroid malignancy may be many years—as many as twenty-five or thirty—despite conservative operations upon the thyroid gland. However, analysis of the survival records in each series shows that postoperative life expectancy depends primarily on the type of malignancy found. The majority of thyroid cancers are either papillary carcinomas or angioinvasive adenomas. These are of a low order of malignancy in that growth is slow, invasion late, and metastasis not common. In our series, 15 out of 25 cases were papillary carcinomas and an additional 6 cases were adenomas associated with blood vessel invasion. Thus 21 out of 25, or 84 per cent of the total cancers had malignancy of low degree both pathologically and biologically. Nonetheless, even this group included one instance of pulmonary metastasis and another instance of local recurrence after one year, both from papillary carcinomas. Four of the papillary carcinomas had

invaded beyond the thyroid at the time of operation, but nevertheless did not recur. Frequently no histologic difference is found between those tumors which are fatal and those which are not. Cancer in the thyroid gland thus exhibits a histologic pattern of carcinoma but behaves biologically in most varied fashion.

Because a large proportion of thyroid malignancies are of low or moderate malignancy, Rogers, Asper and Williams (3) advocate no treatment until there is definite evidence of cancerous behavior such as growth, increased hardness, and adherence to adjacent tissues. On the other hand, Ward (16) found that 80 per cent of patients with a clinical diagnosis or suspicion of thyroid cancer were dead at the end of five years; when cancer was diagnosed at operation, 60 per cent were dead at the end of five years; and when the diagnosis was first made on histo-pathologic examination, only 20 per cent were dead at the end of five years.

Whether malignancy of the thyroid develops from the processes of hyperplasia and involution which result in nodular goiter in a previously normal thyroid gland is difficult to establish. Many malignant tumors of the thyroid undoubtedly remain small for long periods. The mere fact that a tumor of the thyroid has been present for a long time does not, therefore, indicate that it was benign initially. Young (17), in a study of the incidence of carcinoma in thyroidectomized patients in a 5-year period at the University of Oklahoma Hospitals, observed that of the 174 patients in the series, 9 had cancer, an incidence of 5.2 per cent, with 1 small cancer unexpectedly found in a diffuse toxic goiter. Of the 76 patients with thyrotoxicosis, there was an incidence of thyroid cancer of 1.3 per cent. In the 98 patients with nontoxic goiters, 8 cases of cancer were found, an incidence of 8.2 per cent. All of the 8 cases of cancer were palpable as single nodules and were found in the patients with nodular goiter, an incidence of 9.4 per cent. However, these 8 carcinomas occurred in the 44 cases with solitary nontoxic nodular goiter, making an incidence of cancer of 18.2 per cent in this group. None was found in the 10 patients with multinodular goiter.

Because of the higher incidence of cancer in solitary nodules, Young (17) believes that carcinoma is present from the beginning rather than arising as "degeneration" of previously benign nodules. If the latter were true, the higher incidence would occur in the multinodular goiters, because there are more nodules in a given number of patients, and it would also be more common in hyperplastic glands. He concludes, however, that surgical treatment is indicated for all asymptomatic nodular goiters, to remove malignancy already present rather than to prevent future development of cancer within benign nodules.

Experimentally, and perhaps clinically, there is evidence that adenomas may be a factor in the development of malignancy. The relation of hyper-

plasia to neoplasm has received considerable study. Hellwig (18), in a study of the development of thyroid adenomas in experimental animals, observed that adenomas arose as a local hyperplasia of the epithelium and that they represented an intermediate stage between hyperplasia and malignancy. Goetsch (19) studied multiple blocks of tissue from 7 cases of thyroid carcinoma of various types occurring in patients with exophthalmic goiter and demonstrated that miliary adenomas not infrequently existed in the hyperplastic gland. The origin of 3 of these carcinomas was clearly traced to small adenomas. Emmett and Dreyfuss (20) have also stressed the importance of the miliary adenoma in the formation of carcinoma in hyperplastic thyroids.

Lerman, Jones, and Calkins (21) have reported neoplastic changes in the hyperplastic goiters of 2 cretinous brothers. A similar case of cretinism with large goiter was observed by us (22) in a white girl, aged 16, who had a bilateral subtotal thyroidectomy in 1931. Histologically, the resected thyroid tissue showed marked and extensive hyperplasia. Another thyroidectomy in 1936, performed because of marked regrowth of the thyroid remnants, showed marked hyperplasia and adenocarcinoma in the removed specimen.

Knowledge derived from the use of the thiocyanates and thiourea derivatives has yielded significant information on the relation of hyperplasia to neoplasia. Broders and Parkhill (23) point out that in thiocyanate goiter cellular hypertrophy produces a picture comparable microscopically to the goiter of Graves' disease, whereas thiouracil-induced goiters show a cellular hyperplasia with marked mitoses suggestive of carcinoma. Bielschowsky (24, 25) induced benign and malignant tumors in the thyroid glands of rats fed simultaneously with the carcinogenic agent 2-acetylamino-fluorine and allyl thiourea. Griesbach, Kennedy and Purves (26) demonstrated that prolonged hyperplasia of the thyroid produced by a rape-seed diet rich in allylthiourea led to the development of thyroid adenomas. Purves and Griesbach (27) observed metastatic thyroid tumors in the lungs of 2 out of 30 rats maintained for long periods on thiourea; 7 out of 13 tumors of the thyroid produced after twenty months' treatment with thiourea were carcinomatous (28). Money and Rawson (29) produced adenomas in the thyroid of rats treated for long periods with thiouracil alone. Two types of neoplastic changes were observed in the thyroid glands of such rats—one characterized by structures resembling fetal thyroid tissue of the more undifferentiated type, and the other consisting of papillary cystadenomas. In addition, they observed numerous areas which resembled fetal thyroid tissues and growth of undifferentiated tissue projecting into the lumens of follicles in patients subjected to long periods of treatment with thiouracil.

Our data corroborate the experiences of others in demonstrating that a

significant proportion of asymptomatic nodules are thyroid cancers. Surgical exploration is necessary to establish the nature of the nodule or nodules and to extirpate the disease. In this series, bilateral thyroid resections were carried out in only 2 patients and radical neck dissection was not executed in any case (Table 7). Minimal operative procedures have frequently arrested the disease for as long as fifteen years.

The higher incidence of cancer in patients with solitary nodular goiter as compared with multinodular goiter has been stressed to the point where a temporizing attitude towards the patient with asymptomatic multinodular goiter has been advocated. Although in our series the incidence of cancer is somewhat higher in patients with solitary nodules, the difference in incidence is more apparent than real. Table 5 shows the large errors in the clinical estimation of the type of nodularity or enlargement present as compared with the pathologic findings. Since the small, clinically imperceptible nodules may be malignant, many of the cancers that have been listed in the literature on the basis of clinical examination as occurring in solitary nodular goiters have probably occurred in goiters with multiple nodules. For this reason, asymptomatic multinodular goiters should be considered potentially malignant.

The best type of operation for the patient with cancer of the thyroid will vary from total lobectomy to total thyroidectomy and radical neck dissection. The operations utilized in our series have varied from simple excision of the nodule to bilateral subtotal thyroidectomy. One patient with Grade III epidermoid carcinoma, treated by a left subtotal thyroidectomy, died fifteen months later of disseminated metastases, even though at operation there was no local extension of the thyroid tumor nor evidence of metastases. It is possible that this patient might have lived longer if a more radical operation had been performed. Local recurrences have been observed in 2 patients (Cases 21 and 23). These patients had excision of nodules at the primary operation, and are living and free of thyroid malignancy following secondary removal of the local recurrence. In 1 patient (Case 17) who had total lobectomy, a functioning metastasis developed in the right lung, and was successfully treated by I^{131}. It is not possible to determine whether neck dissection in this patient would have prevented a pulmonary metastasis. The local recurrence in the other 2 patients, however, might have been prevented by more extensive local surgery at the first operation. In view of this, we feel that total thyroidectomy and a radical neck dissection should be performed in those instances in which there is local extension or lymph node involvement. In all other cases, the operation should consist of either total lobectomy or total thyroidectomy, depending on the character of the nodule or nodules

and their extent. Total thyroidectomy should be performed in all instances in which cancer is known to exist at operation, if future treatment with radioactive iodine is contemplated.

Postoperative radiation has proved of doubtful value, since recurrences or metastases have developed in spite of external radiation following the primary operation (Cases 7, 17, 21 and 23). In recent years, we have used external radiation only in cases in which inadequate surgery was performed and secondary operation was not possible.

The complications of thyroidectomy in this series are listed in Table 6. The important complications were recurrent laryngeal nerve injury (as determined by postoperative laryngoscopic visualization of the vocal cords), myxedema, and tetany. Injuries to the recurrent laryngeal nerves were fairly frequent and were significantly related to the type of operation. Simple excision of nodules and total lobectomy or thyroidectomy produced a higher incidence of nerve injury than subtotal thyroidectomy, whether unilateral or bilateral. Nerve injury has occurred in cases in which the recurrent laryngeal nerve was carefully demonstrated, so that routine exposure of these nerves does not necessarily protect against vocal cord paralysis. Tetany and myxedema, on the other hand, occurred only with bilateral operations, with an incidence comparable to that in most reported series. The only operative mortality occurred in an elderly patient with cancer of the thyroid, who died of heart failure fourteen days after a biopsy of the thyroid.

SUMMARY AND CONCLUSIONS

1. A study of the treatment of 200 patients with nodular goiter is presented.

2. In 190 patients, it was possible to determine the exact degree and extent of the nodularity. In 137 of these, the goiter was judged clinically to contain a solitary nodule, but complete examination of the excised specimen revealed that 43 of these goiters were multinodular and 94 were uninodular; also, both single and multiple nodules were found in 18 clinically non-nodular thyroid glands. This throws considerable doubt on the clinical differentiation of solitary from multinodular goiter.

3. The incidence of malignant neoplasms in the entire series was 12.5 per cent; 14.4 per cent of the solitary nodules were malignant neoplasms. In 10.4 per cent of the patients with multinodular goiters, malignant neoplasms occurred in one or more of the nodules.

4. Thyroid cancer occurred as an unexpected finding in patients with asymptomatic nodular goiter.

5. Twenty-five patients with malignancy of the thyroid were followed

for periods of from one to seventeen years. Twenty-one of these patients had papillary carcinoma or angioinvasive adenoma. Only 2 patients died during a follow-up period which averaged seven years.

6. Routine radical neck dissection in thyroid cancer does not appear to be justified. Adequate surgery should consist of unilateral total lobectomy or bilateral thyroidectomy, depending upon the nature and extent of the lesion. Radical neck dissection is indicated when there is transcapsular spread.

7. Injuries to the recurrent laryngeal nerves have a significant incidence in the surgery of nodular goiter, and are related to the type of operation performed.

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THE PRESENT STATUS OF THE TREATMENT OF TOXIC GOITER*

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THIS paper presents a brief summary of the various methods of treatment of toxic goiter that are available at the present time and the current attitude toward them.

Until a few years ago it was generally agreed that the best method of treatment was a subtotal thyroidectomy after adequate preoperative preparation with iodine, a high-caloric diet and rest. Various details had been perfected so much that the mortality from surgical procedures had dropped to about 1 per cent in leading clinics. In the past few years treatment has been greatly modified by the use of antithyroid drugs and radioactive iodine.

There are five methods of treatment available at the present time:

1. A subtotal thyroidectomy after adequate preoperative preparation with propylthiouracil, iodine and a high-caloric diet.
2. Medical management with antithyroid drugs.
3. Medical management with radioactive iodine.
4. Roentgen ray treatment of the thyroid.
5. Roentgen ray treatment of the pituitary.

SUBTOTAL THYROIDECTOMY AFTER ADEQUATE PREOPERATIVE PREPARATION

In spite of the advances which have occurred, it is the consensus that the best method of treatment of toxic goiter in most instances is still a subtotal thyroidectomy after adequate preoperative preparation. However, the method of preoperative preparation has been modified. By the use of propylthiouracil or some other suitable antithyroid drug it is possible to reduce the basal metabolism to normal and eliminate the thyrotoxicosis before operative procedures are carried out. As a result, the risk of operation is no greater than in patients with nontoxic goiters.

There is some difference of opinion as to whether or not propylthiouracil

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should be given to all patients preoperatively. In some clinics this drug is used only in patients who have the disease in severe form and not in patients who have it in moderately severe or mild form. In other clinics it is used routinely in all patients, regardless of the degree of thyrotoxicosis. The mortality from subtotal thyroidectomy appears to be about the same with both methods of preparation. By giving the drug only to those patients who have the disease in severe form, the frequency of drug reactions is greatly reduced.

Most ambulatory patients may be prepared for operation at home, thereby reducing the cost of hospitalization. The usual method of preoperative preparation is as follows:

1. Propylthiouracil is administered until the basal metabolism drops to the normal level. During its administration the patient is followed carefully in order to detect drug reactions as soon as they occur.
2. Propylthiouracil is commonly discontinued for from four to seven days before operation and iodine substituted for it. This procedure is employed to eliminate the danger of a drug reaction at the time of operation and to reduce the vascularity of the gland by inducing storage of colloid. Some clinics continue propylthiouracil up to the time of operation but supplement it with the administration of iodine for about one week before operative procedures are carried out.
3. Throughout the whole preoperative period the patient is given a diet sufficiently high in calories to cause a gain in weight.
4. Iodine is continued for at least one week postoperatively, to control residual thyrotoxicosis that may be present in a small percentage of patients.

With this method of preoperative preparation the mortality from subtotal thyroidectomy for toxic goiter in leading clinics has dropped to 0.1 per cent, which means that the danger of operation in good hands is very slight.

There are two major complications of thyroidectomy that have not been eliminated, namely, the development of parathyroid tetany and paralysis of one or both vocal cords. Parathyroid tetany still occurs in about 1 per cent of patients and paralysis of one vocal cord in about the same number. Paralysis of both vocal cords is much less common but is a serious complication when it occurs.

Postoperative pneumonia is usually caused by factors incidental to operation and can be prevented in most instances by careful management of the patient. Pulmonary embolus is uncommon. Coronary occlusion occasionally develops in patients with disease of the coronary arteries.

The condition of the patient at the time of operation and the skill of the

surgeon continue to be the two major factors which determine operative mortality. The first is more important than the second. A subtotal thyroidectomy for toxic goiter is an elective operation and should not be performed unless the condition of the patient warrants it. For example, no operative procedure should be carried out until at least two weeks have elapsed after an upper respiratory infection has disappeared.

MEDICAL MANAGEMENT WITH ANTITHYROID DRUGS

As data have accumulated, it has become apparent that a permanent remission in toxic goiter can be induced with antithyroid drugs in only about 50 per cent of patients. Relapses are common after the basal metabolism has been maintained at the normal level for one year or longer. The low percentage of permanent remissions and the danger of drug reactions make the routine medical management of toxic goiter with antithyroid drugs an impractical procedure.

They still have a place, however, in the medical management of patients who for some reason present a poor operative risk, in areas where radioactive iodine is not available. The probability of producing a permanent remission with radioactive iodine is so much greater than with antithyroid drugs that the former may replace the latter for medical management.

MEDICAL TREATMENT WITH RADIOACTIVE IODINE

In patients with exophthalmic goiter radioactive iodine will induce a permanent remission in fully as high a percentage of patients as will a subtotal thyroidectomy. It is easy to administer because it is effective by mouth and its use is rarely attended by any serious reaction.

The reason that it has not replaced subtotal thyroidectomy in the treatment of exophthalmic goiter is that it is still uncertain whether or not radioactive iodine causes carcinoma of the thyroid. The data available up to the present time indicate that it may not; but many more years will have to elapse before any definite conclusion can be drawn on this point. The medical management with radioactive iodine of patients with exophthalmic goiter over 55 years of age would appear to be justified, because the data that have been collected indicate that there is little likelihood of carcinoma of the thyroid developing in these patients during their expected life span.

Radioactive iodine is also of value in the medical management of patients who have a persistence or recurrence of exophthalmic goiter, although in younger individuals many clinics still prefer to carry out a second thyroidectomy after adequate preoperative preparation. Medical management with radioactive iodine is indicated in any patient in whom the risk of operation is too great to warrant a subtotal thyroidectomy as,

for example, in patients with heart failure who fail to respond to all known methods of treatment.

In patients who have nodular goiter with hyperthyroidism the consensus is that a subtotal thyroidectomy should be carried out after suitable preoperative preparation, regardless of the age. The reason for this point of view is that the incidence of carcinoma is slightly higher in patients with nodular goiter and hyperthyroidism than it is in patients with exophthalmic goiter, and it is possible that the risk of inducing carcinoma with radioactive iodine is higher in patients with nodular goiter. There is one other factor which renders the use of radioactive iodine less desirable in patients with nodular goiter, namely, the fact that nodular goiters with hyperthyroidism do not take up a very large amount of radioactive iodine and therefore much larger doses must be employed to control the thyrotoxicosis.

It should be pointed out that carcinoma is not commonly encountered in patients with toxic goiter, regardless of the type.

ROENTGEN RAY TREATMENT OF THE THYROID

Before the introduction of iodine in preoperative preparation there was some justification for medical management of toxic goiter by roentgen ray treatment of the thyroid, because of the risk of operation. After the introduction of iodine, the use of roentgen ray therapy was limited largely to the treatment of patients who presented a poor operative risk.

At the present time there is little indication for the use of roentgen ray irradiation of the neck, because radioactive iodine works so much better and brings the effective rays directly in contact with the thyroid cells. The danger of burning the skin of the neck is eliminated.

ROENTGEN RAY TREATMENT OF THE PITUITARY

A few years ago we found that in a small series of patients roentgen ray treatment of the pituitary would produce a remission in toxic goiter in about one third of the patients. This observation is of interest from the standpoint of the mechanism involved in the production of thyrotoxicosis but at the present time has little therapeutic application. The percentage of remission with this form of therapy is too low to warrant its routine use.

SUMMARY

1. The best method of treating toxic goiter in most instances is still a subtotal thyroidectomy after adequate preoperative preparation.

2. When medical management is indicated, radioactive iodine appears to be preferable to antithyroid drugs.

3. The most important use of antithyroid drugs appears to be in the

preoperative preparation of patients who have the disease in severe form.

4. Radioactive iodine may be employed for medical treatment of exophthalmic goiter in patients over 55 years of age. It is also of value in the treatment of persistence and recurrence of exophthalmic goiter but it is uncertain whether it should be used for this purpose in younger individuals.

5. Sufficient time has not yet elapsed to be certain whether or not radioactive iodine causes carcinoma of the thyroid. It is primarily for this reason that its routine use in the medical management of exophthalmic goiter at all ages is not warranted at present.

6. The consensus is that in most patients who have nodular goiter with hyperthyroidism the best method of treatment, regardless of the age, is a subtotal thyroidectomy after adequate preparation.

7. Radioactive iodine is not as effective in the control of thyrotoxicosis in patients with nodular goiter as in those with exophthalmic goiter.

LETTERS TO THE EDITOR

EFFECT OF X-RADIATION ON 17-KETOSTEROID EXCRETION

To the Editor:

Lawrence has reported studies on the excretion of 17-ketosteroids by dogs after whole-body x-radiation (*Endocrinology* **45**: 383, 1949). He found a significant increase in the urinary excretion of neutral 17-ketosteroids, which occurred approximately one week after a single treatment with 350 r.

We have not been able to duplicate this study in our laboratory. The normal excretion of 17-ketosteroids in a series of 8 dogs was less than 1 mg./day and no increase was noted after 350 r of whole-body x-radiation. There was no increased excretion of 17-ketosteroids by 2 dogs given ACTH in doses of 15 mg. every six hours for two days.

Glenn and Heftman (*Proc. Soc. Exper. Biol. & Med.* **77**: 147, 1951) have reported a similar low excretion (1 mg./day) of 17-ketosteroids by dogs, and no increase following acute doses of ACTH.

This study of Glenn and Heftman, which is in agreement with our own, concludes that the larger amounts of 17-ketosteroids reported for dogs are probably the result of interference with the colorimetric assay by urinary chromogens.

Since the Lawrence data are often quoted in support of the hypothesis of adrenal stimulation by radiation, it should be noted that:

1. The dog adrenal does not respond to ACTH with increased excretion of 17-ketosteroids.
2. Single whole-body x-radiation did not result, in our experiment, in any increase in 17-ketosteroid excretion.

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DIAGNOSTIC ERRORS BASED ON SINGLE ASSAY OF I^{131} UPTAKE AFTER INTRAVENOUS TRACER DOSE

To the Editor:

Several authors have proposed the use of intravenous injection for administering the tracer dose of I^{131} in determinations of thyroid uptake

(Dargent, M. *et al.*: *Lyon Chir.* **45**: 287, 1950. Fauvert, R.: Les acquisitions medicales recentes. Paris, Flammarion, 1951.) Certainly, provided the solutions are carefully sterilized and distilled water and neutral glass are used, this technique of administration is harmless, rapid and convenient.

Kriss (*J. Clin. Endocrinol.* **11**: 289, 1951) claims that this method is especially useful in the diagnosis of hyperthyroidism, since sometimes the thyroid uptake is higher one hour after the administration than twenty-four hours afterward.

It should be pointed out, however, that a single determination of the uptake in the first hour after intravenous administration of the isotope may, in many cases, cause errors in the evaluation of thyroid function.

In strumitis, in Simmonds' disease, and especially in endemic cretinism with goiter, the thyroid uptake of I^{131} may increase very slowly and limit itself to very low values in the first hour after the isotope is administered intravenously, whereas the uptake may reach normal values twenty-four to thirty-six hours later. We have observed in cases of cretinism with goiter, uptakes of 5–7 per cent in the hour following the administration of 40 microcuries of I^{131}; the uptakes increased to 15–20 per cent twenty-four hours afterwards.

It is, therefore, desirable that in every case an uptake control value be determined after twenty-four hours.

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A THEORY TO EXPLAIN ANOVULATORY CYCLES

TO THE EDITOR:

A clear understanding of the complex mechanism in regard to anovulatory cycles is still lacking. In spite of the numerous suggestions as to the possible mechanism there has been no generally accepted explanation for the failure of ovulation in some women during the reproductive age. It is believed by many that the presence of multiple follicles in the ovaries in some patients may have some bearing on this failure. No specific elucidation of a possible missing link in the hypothalamo-pituitary-ovarian mechanism has been presented.

I wish to submit the following theory in an attempt to shed some light upon the *modus operandi* in anovulatory cycles.

The stimulating factor that precipitates the menarche in the young female is still obscure. To the question of what initiates any physiologic function in the human body, the answer would usually be, "a nervous stimulus." Since the hypothalamus is part of the central nervous system,

one may postulate that the menarche is initiated by it, perhaps aided by one or more of the five senses of the human being. This theory would explain the awakening activity of the hormonic factors, which are dormant from the embryonic stage to the onset of the menarche, in contrast to the chromosomic factors which manifest themselves in the early embryonic stage of development. There appears to be a well organized sequence. The potentialities of hormonic potency are there, but the awakening appears only at a certain age. Could it be that it takes a certain number of years before the seed sprouts its stem above the ground?

Let me briefly describe the generally accepted facts about the workings of the follicular apparatus of the ovary in relation to the hypothalamo-pituitary mechanism. At the menarche, the primordial follicles of the ovary are stimulated by the follicle-stimulating hormone (FSH) of the anterior pituitary gland and a certain number of these follicles start out in a race, so to speak, each trying to become the "chosen" Graafian follicle. Whether these follicles are present in a constant number at birth or develop during the reproductive stage is not important to the point under discussion. Usually only one follicle succeeds in becoming the mature follicle and the rest wither away. This Graafian follicle has a dual purpose, namely, to form a mature ovum and to produce the alpha-estradiol which is necessary in order to prime the endometrium and its associated reproductive organs. The reproductive organs are further acted upon by progesterone to complete the nidatory receptacle and its supporting mechanism. Nature has worked such a perfect plan that by the time the ovum is ready for discharge, the concentration of estrogen has reached its peak, thus inhibiting the further secretion of FSH and releasing the luteinizing hormone (LH) from the anterior pituitary. The LH together with the third hormone, luteotropin, causes the metamorphosis of the ruptured Graafian follicle into a corpus luteum, the latter producing progesterone as well as estrogen.

On the other hand, if we assume that the inhibitory factor which normally arrests the remaining follicles in the early stage (aside from the destined Graafian follicle) is inactive, many follicles would continue to grow and secrete their share of estrogen, and it would therefore take less time to bring the concentration of the estrogen to its peak. If this sequence occurs, no Graafian follicle would have the opportunity to mature, since the FSH would be prematurely inhibited. A situation of anovulatory cycles would be thus created.

This theory could be applied in the early menarche as well as in the early menopause. It would explain the irregular menses at both these periods of life, since the menstrual flow at these times is a result of estrogen-with-

drawal bleeding. Such an abnormal mechanism is assumed by our present methods of therapy, *e.g.*:

1. Small doses of x-rays to the ovaries cause the follicles to be destroyed. The effect of x-rays on the pituitary results in further secretion of FSH despite the inhibition of the high concentration of estrogen in the blood stream, thus permitting the development of a mature Graafian follicle.

2. Resection of the ovary accomplishes the same result. It eliminates the multiple follicles and allows a new crop to start out with the hope that the normal mechanism will then prevail.

3. The few good results obtained with the use of the pituitary-like hormone extracts (gonadotropins) can be explained on the same basis.

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The Endocrine Society

THE 1952 ANNUAL MEETING

The Thirty-fourth Annual Meeting of The Endocrine Society will be held at the Palmer House, Chicago, Illinois, Thursday, Friday, and Saturday, June 5–6–7, 1952.

The Local Chairman of Arrangements is Dr. Willard O. Thompson.

All Scientific Sessions will be held in the Palmer House. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 7, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All members are urged to make hotel reservations immediately, inasmuch as the hotels expect to be filled to capacity. Make reservations directly with the Palmer House, advising time of arrival and departure date. Make your reservations now and avoid disappointment.

Those wishing to present papers, which will be limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Evelyn Anderson, National Institutes of Health, Bethesda 14, Maryland, not later than *March 1, 1952. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and journals of the Association.* The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.
2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each nonmember-author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of nonmembers who are introduced, *i.e.*, who are not collaborators with member-authors,

are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.

Line 3. Institution of origin and city in which institution is located.

3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proofreading by authors.

1952 AWARDS AND FELLOWSHIPS

Fellowships

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

This Fellowship will not be awarded in 1952, so that it may accumulate toward a $5,000 Fellowship to be awarded in 1953.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected for the Fellowship in 1949. The 1950 Award was made to Dr. Lawrence E. Shulman.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

Awards

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb & Sons Award of $1,000 was established in 1939, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; in 1950 to Dr. C. N. H. Long; and in 1951 to Dr. J. B. Collip. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

Announcement of Increase in the E. R. Squibb and Sons Award

This Award has been increased to the amount of *$2,500* beginning with the 1952 Award.

(The Society expresses its sincere gratitude to E. R. Squibb and Sons for its interest in furthering research in this important field of medicine.)

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator not more than thirty-five years of age in the field of clinical or pre-clinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; and in 1951 to Dr. Albert Segaloff. The Award is for $1,200. If, within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma, and returned to him *not later than March 1, 1952.*

The American Goiter Association

THE 1952 ANNUAL MEETING

The next Annual Meeting of the American Goiter Association will be held in St. Louis, Missouri, May 1, 2 and 3, 1952, at the Hotel Chase. All those who plan to attend are urged to make hotel reservations immediately.

VAN METER PRIZE AWARD FOR 1952

The American Goiter Association again offers the Van Meter Prize Award of three hundred dollars and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. Providing essays of sufficient merit are presented in competition, the Award will be made at the annual meeting of the Association which will be held in Saint Louis, Missouri, May 1, 2 and 3, 1952.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double-spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, *not later than March 1, 1952*. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.